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Title of Thesis: "Effects of Clozapine and Alprazolam on Cognitive Deficits and Anxiety-like Behaviors in a Ketamine-Induced Rat Model of Schizophrenia"

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ABSTRACT

Title of Thesis: "Effects of Clozapine and Alprazolam on Cognitive Deficits and Anxiety-like Behaviors in a Ketamine-Induced Rat Model of Schizophrenia"

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Schizophrenia is a debilitating mental illness that affects approximately 2.2 million Americans (1% of the population) each year. Despite the large number of people affected by schizophrenia, there is no known cure for this disorder but antispychotics help to manage the symptoms of schizophrenia. Unfortunately, antipsychotic medications are frequently accompanied by debilitating side effects (*e.g.,* extrapyramidal side effects, tardive dyskinesia) and low compliance. Alternative pharmacological treatment options are needed to improve the treatment and quality of life for individuals suffering from schizophrenia.

This doctoral research project examined the hypothesis that the cognitive deficits associated with schizophrenia can be medicated indirectly using anxiolytics, drugs that decrease anxiety, based upon a proposed relationship between anxiety and cognitive disruptions in schizophrenia. The current research also evaluated the validity and potential usefulness of ketamine

administration to create a novel animal model that includes symptoms of schizophrenia and anxiety.

Two experiments were conducted to address these goals. Experiment #1 examined the effects of clozapine (an antipsychotic), alprazolam (an anxiolytic), and a combination treatment on ketamine-induced cognitive disruptions in prepulse inhibition (PPI) of the acoustic startle reflex and passive avoidance. Experiment #2 examined the effects of clozapine, alprazolam, and a combination treatment on ketamine-induced anxiety-like behaviors on the elevated plus maze, open field test, and social interaction test.

The major findings of the study were: (1) ketamine administration caused cognitive disruptions in PPI as well as passive avoidance; (2) only clozapine attenuated the cognitive disruptions caused by ketamine, and only in the PPI measure; (3) ketamine administration caused an increase in anxiety-like behaviors on the EPM, open field locomotor activity, and social interaction; (4) only alprazolam decreased the ketamine-induced increases in anxiety-like behaviors, and only in the measure of social interaction. The findings failed to support the hypothesis that ketamine-induced cognitive deficits could be attenuated with anxiolytic medications. In addition, these findings suggest that ketamine administration may provide a useful, but limited, model of concurrent symptoms of schizophrenia and anxiety.

Effects of Clozapine and Alprazolam on Cognitive Deficits and Anxiety-like Behaviors in a Ketamine-Induced Rat Model of Schizophrenia

by

Jennifer M. Phillips

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INTRODUCTION

Overview

Schizophrenia is a debilitating mental illness that affects approximately 2.2 million Americans or 1% of the population each year (American Psychiatric Association, 2000; Kendler, Gallagher, Abelson, & Kessler, 1996; Regier, et al., 1993). Despite the large number of people affected by schizophrenia, there is no known cure for the disorder and schizophrenia treatment has low efficacy and low compliance rates. Use of the most common treatment, antipsychotic medications, is frequently accompanied by debilitating side effects and low compliance. Alternative pharmacological treatment options are needed to improve the treatment and quality of life for the individuals suffering from this disorder. The current research examined the hypothesis that the cognitive deficits associated with schizophrenia can be medicated indirectly using anxiolytics, drugs that decrease anxiety – medications that are significantly better tolerated than antipsychotic medications. The current research also evaluated the validity and potential usefulness of a novel animal model combining symptoms of schizophrenia and anxiety. These studies examined the effects of a standard pharmacological treatment for schizophrenia, the antipsychotic clozapine, and a non-traditional pharmacological treatment, the anxiolytic alprazolam, as well as a combination treatment, on cognitive deficits and anxiety in a ketamine-induced animal model of schizophrenia.

Schizophrenia

Description

According to the American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders IV - Text Revision* (DSM-IV-TR), schizophrenia is a psychotic disorder characterized by the presence of positive and negative symptoms associated with marked social and occupational dysfunction (APA, 2000). **Table 1** presents a list of DSM-IV-TR diagnostic criteria for schizophrenia. Positive symptoms, defined as an excess or distortion of normal functions, include delusions, hallucinations, and disorganized speech and behavior. Common negative symptoms of schizophrenia, defined as restrictions in the

Table 1. DSM-IV-TR criteria for schizophrenia (abbreviated)

A. Characteristic symptoms: Two (or more) of the following, each present for a significant portion of time during a 1-month period:
   1. delusions
   2. hallucinations
   3. disorganized speech (*e.g.,* frequent derailment or incoherence)
   4. grossly disorganized or catatonic behavior
   5. negative symptoms, *i.e.,* affective flattening, alogia, or avolition

B. Social/occupational dysfunction: For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset.

C. Duration: Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms that meet Criterion A and may include periods of prodromal or residual symptoms.

D. Schizoaffective and mood disorder exclusion

E. Substance/general medical condition exclusion

F. Relationship to a pervasive developmental disorder: Additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month.

(Based on *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR 4th edition Text Revision*, APA, 2000)

range and intensity of normal behaviors, are affective flattening, alogia, and avolition (APA, 2000).

Schizophrenia is also characterized by cognitive deficits that are believed to be related to, but separate from, the negative symptoms of the disorder (Harvey et al., 1996; USDHHS, 1999). Multiple cognitive domains are affected in schizophrenics, including attention and vigilance, information processing, executive function, language skills, and working memory (Bilder et al., 1992; Riley et al., 2000; Sharma & Antonova, 2003). Although this cognitive dysfunction is often described as a symptom of schizophrenia, certain theories posit that deficits in fundamental cognitive processes, such as attention and memory deficits, may be at the core of the disorder, rather than simply a symptom of schizophrenia (Andreasen, 1997a; 1997b; Andreasen et al., 1996). Regardless of their exact role in the disorder, these cognitive deficits contribute substantially to the functional impairments associated with schizophrenia (Green, 1996) and are a frequent target for therapeutic interventions.

Prevalence

Based upon data from the Global Burden of Disease Project, the World Health Organization (WHO, 2000) reports a 0.4 percent worldwide point prevalence of schizophrenia. The general one-year prevalence in adults age 18-54 is estimated to be 1.3 percent, and in the United States, schizophrenia affects approximately 2.2 million Americans each year (APA, 2000; Kendler et al., 1996; Regier et al., 1993; USDHHS, 1999).

Historically, it has been reported that men are more likely to develop schizophrenia than women. More recently though, this belief has been challenged by new, more standardized data that support approximately equal prevalence rates for men and women (Goldner, Hsu, Waraich, & Somers, 2002). Although there is some debate as to prevalence differences between the sexes, it is generally accepted that average age of onset is younger for men (early - mid 20s) than for women (late 20s) (APA, 2000; USDHHS, 1999) and that women tend to have a less severe course of illness (APA, 2000; Tamminga, 1997; USDHHS, 1999).

Costs of Schizophrenia

Like many other severe, chronic illnesses, schizophrenia presents a large economic burden for society. In the 1990s, schizophrenia cost the United States an estimated $65.1 billion dollars annually in direct and indirect costs (Wyatt, Henter, Leary, & Taylor, 1995). The Health Economics Resource Center (HERC), a Veterans' Administration (VA) Health Services Research and Development Center, estimated that the average cost for VA-related health care in patients diagnosed with schizophrenia was $14,385 per patient, per year (HERC, 2002). Large proportions of these expenses are directly attributable to treatment costs and lost productivity resulting from a lack of treatment, low treatment efficacy, and compliance.

In addition to economic costs, schizophrenia also results in significant decreases in lifespan and quality of life. The average lifespan of schizophrenics

is 10 years shorter than that of non-affected individuals (WHO, 2000) and a multi-country study reported psychotic disorders such as schizophrenia to be the third most disabling condition among all psychological and medical disorders (Üstün et al., 1999). Schizophrenics are also at a much greater risk of suicide, with studies reporting as much as 30% of the schizophrenic population attempting suicide at least once during the course of their illness (Radomsky, Haas, Mann, & Sweeney, 1999) and 10% of schizophrenics successfully completing a suicide attempt (Caldwell & Gottesman, 1990). Treatment is a logical method to reduce such negative outcomes, but compliance with the most common forms of treatment for schizophrenia is consistently low.

Etiology of Schizophrenia

The cause of schizophrenia is not known although many theories have been proposed to explain the disorder. Most of these theories rely upon a significant role of genetic predisposition in the development of schizophrenia. Biological relatives of schizophrenics are 10 times more likely to develop the disorder than the general population (Kety et al., 1994). This difference translates into a 5-10% lifetime risk for first-degree relatives of schizophrenics (USDHHS, 1999). A monozygotic twin of a schizophrenic is at an even greater risk of developing the disorder than are other biological relatives. A review of five twin studies by Gottesman and Shields (1976) found a concordance rate of 35-58% in monozygotic twins and a concordance rate of 9-26% in dizygotic twins. The findings from twin studies suggest that genetics play a significant role in the

development of schizophrenia. However, the substantial discordance in monozygotic twins in the development of schizophrenia suggests that genetics alone cannot account for who develops the disorder. There are other factors, possibly environmental, that affect development of the disorder as well. It is likely an interaction between genetic and environmental influences that ultimately determines the onset of schizophrenia.

Neurodevelopmental models have been proposed to explain the development of schizophrenia based on the premise that the origins of schizophrenia are in abnormal brain development that originates during intrauterine fetal development (Murray & Lewis, 1987). The cause of the abnormal development is commonly speculated to be a type of stressor with candidates ranging from maternal poverty (Cohen, 1993) and prenatal depression (Jones, Rantakallio, Hartikainen, Isohanni, & Sipila, 1998) to viral exposure (Kirch, 1993; Torrey, 1991; Torrey & Peterson, 1976) and Rh factor incompatibility (Hollister, Laing, & Mednick, 1996). Infants of low birth weight and premature birth are at higher risk for developing schizophrenia (Jones et al., 1998) as are infants experiencing difficult deliveries and delivery complications (Jones & Cannon, 1998). There is debate as to whether developmental and fetal complications such as these are reflective of, or actually the cause of, disrupted development in schizophrenics (Lipska & Weinberger, 1997).

Neuropathology in Schizophrenia

Multiple gross brain abnormalities have been noted in schizophrenics including ventricular enlargement and decreased cortical volume and unusual cortical laterality with disruptions limited to the left hemisphere (Bruton et al., 1990; Harrison, 1999). These abnormalities have been correlated with both the positive and negative symptoms of schizophrenia. Positive symptoms are frequently linked to temporal lobe dysfunction (*e.g.,* McGuire et al., 1998), whereas negative and cognitive symptoms are commonly associated with prefrontal lobe dysfunction (Capleton 1996; USDHHS, 1999).

Gliosis, the production of a dense fibrous network of neuroglia, also has been linked to schizophrenia. Stevens (1982) reported that 70% of schizophrenics in one study evidenced gliosis in diencephalic regions of the brain. Subsequent work has failed to replicate this finding in many cases, and suggests that any gliosis may be a result of other neuropathy associated with schizophrenia rather than a result of the disorder itself (Harrison, 1999).

Neurochemistry in Schizophrenia

Disruptions in several neurotransmitters, including dopamine, serotonin, and glutamate systems, are associated with schizophrenia and psychotic symptoms.

Dopamine: Excessive dopaminergic activity and sensitivity through various mechanisms including increased dopamine release, decreased dopamine reuptake, increased receptor density and availability, have been implicated in schizophrenia, particularly in connection with positive symptoms of

the disorder and in the mesolimbic dopamine pathway. Neuroleptic medications that reduce dopamine levels also reduce the appearance of psychotic symptoms in schizophrenic patients. Drugs that increase dopamine levels, such as amphetamine, also increase the occurrence of psychotic symptoms in schizophrenics and induce psychosis-like episodes in non-schizophrenics (Stahl, 2002). Finally, dopamine receptor density, particularly of the D_2 variety, is increased in schizophrenics. There is also speculation that dopamine levels may be deficient in certain areas of the brain in schizophrenics, specifically the mesocortical dopamine pathway, which may contribute to negative symptoms of the disorder (Stahl, 2002).

Serotonin (5-HT): The connection between serotonin and schizophrenia is based largely on the 5-HT_{2A} receptor. Atypical antipsychotic medications have a high affinity for this particular receptor (Harrison & Burnet, 1997) and polymorphisms for the gene encoding 5-HT_{2A} receptors are reportedly a minor risk factor for developing schizophrenia (Williams, McGuffin, Nothen, & Owen, 1997). Additionally, lowered 5-HT_{2A} receptor expression in the frontal cortex of schizophrenics has been reported (Harrison, 1999). Serotonin may be acting by itself or in connection with dopamine to produce symptoms of schizophrenia (Harrison, 1999).

Glutamate: The non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP) has been used to model symptoms of psychosis

in humans (Abi-Saab, Souza, Moghaddam, & Krystal, 1998; Geyer, Krebs-Thompson, Braff, & Swerdlow, 2001; Marcotte, Pearson, & Srivastava, 2001), lending support to the involvement of glutamate in the disorder. Glutamatergic markers are decreased and there is reduced expression of certain NMDA receptors in the medial temporal lobe of schizophrenics, although patterns differ in other brain regions (Harrison, 1999). Glutamate also may be acting in connection with dopamine to produce dysfunction in schizophrenics (Carlsson & Carlsson, 1990).

Treatment of Schizophrenia

Schizophrenia is most commonly treated pharmacologically using a variety of antipsychotic medications that act in ways that are consistent with the theorized neurochemical dysfunctions of the disorder. The antipsychotic properties of early medications used to treat schizophrenia were discovered serendipitously. The first antipsychotic, chlorpromazine, was synthesized in 1950 as an antihistamine. Its antipsychotic properties were discovered soon after and it was first administered to a psychiatric patient as a treatment for schizophrenia in 1952 (Lehmann & Ban, 1997). Chlorpromazine and various drugs with similar mechanisms are known as neuroleptics or typical antipsychotics. They all share the common mechanism of dopamine 2 (D_2) receptor blockade in the mesolimbic dopamine pathway and were instrumental in uncovering the role of dopamine in schizophrenia. D_2 receptor blockade reduces the dopaminergic hyperactivity in the mesolimbic dopamine pathway that is

believed to cause certain positive symptoms of schizophrenia. Consequently, conventional antipsychotics and neuroleptics cause a reduction in positive symptoms. The effects of neuroleptics are not specific to the areas of dopamine hyperactivity. Decreases in dopamine activity in other areas of the brain are believed to be responsible for the debilitating side effects, including tardive dyskinesia and extrapyramidal side effects, associated with the neuroleptic drugs (Stahl, 2002). Conventional antipsychotics and neuroleptics were the pharmacological treatments of choice until the introduction of the newer atypical antipsychotics, a group of medications that have different mechanisms but equal efficacy in treating psychotic disorders. The older drugs then fell out of favor, largely as a result of their many negative and debilitating side effects and difficulty in treating negative symptoms.

The first atypical antipsychotic to gain credibility as an improved antipsychotic medication was clozapine in the early 1980s when it was shown to have significantly fewer side effects, equal efficacy in standard schizophrenics, and a higher efficacy in treatment-resistant schizophrenics (Lehman & Ban, 1997). These effects result from antagonism of serotonin 5-HT_{2A} and dopamine D_2 receptors. Serotonin inhibits dopamine release in the brain, but does so differentially in the various dopamine pathways. This differential effect, combined with the D_2 receptor antagonist properties in atypical antipsychotics, allows for greater dopamine availability in areas where dopamine is decreased in schizophrenia and decreases dopamine availability in areas of dopamine hyperactivity in schizophrenics (Stahl, 2002). The location-specific effects of

these newer drugs also help to reduce the number and intensity of negative side effects, although there are still associated side effects. Multiple new atypical antipsychotics have been introduced since the 1980s and are now considered the first-line medications for schizophrenia treatment based upon their efficacy and improved side effect profile.

Although the atypical antipsychotics present a more desirable treatment option than the neuroleptic medications, problems of compliance still exist. A study by Docherty and colleagues (2002) of more than 600 schizophrenic outpatients reported no differences in adherence between patients on typical and atypical antipsychotics. Another study reported that although patients on atypical antipsychotics had higher medication adherence than did those on conventional neuroleptics, there was only a 55% adherence rate over 12 months for patients taking the atypical medications (Dolder, Lacro, Dunn, & Jeste, 2002). The reason for such low medication compliance in schizophrenics is not well understood, but suggests that alternative treatments should be examined.

Anxiety in Schizophrenia

One aspect of schizophrenia that makes it a complicated disorder to study and treat is its varied symptomatology. In addition to positive and negative symptoms and cognitive deficits, schizophrenics frequently experience anxious symptomatology and phobias (APA, 2000). The lifetime prevalence of anxiety disorders in individuals with schizophrenia is estimated to be 30-45% (Cosoff & Hafner, 1998; Dixon et al., 2001), with symptoms of anxiety occurring in more

than half of all schizophrenics (Tollefson & Sanger, 1999). Anxiety may occur during one, some, or all of the phases of schizophrenia including the prodromal phase, psychotic or break periods, and remission or relapse phases. The nature of anxiety in schizophrenia, whether it is a true symptom of schizophrenia, a response to negative social reactions to indivudals with disorder, or even a side effect of medications, is uncertain.

Anxiety further impairs schizophrenics' functioning and negatively impacts treatment compliance. Symptoms of anxiety are related to higher rates of relapse, disability, and suicide in schizophrenics (Tollefson & Sanger, 1999). Importantly, anxiety also has been implicated as a potential mediator or cause of core cognitive symptoms of schizophrenia, including delusions, attentional biases, and attentional dysfunctions (Braunstein-Bercovitz, 2000; Braunstein-Bercovitz, Rammsayer, Gibbons, & Lubow, 2002; Tollefson & Sanger, 1999).

Anxious symptomatology needs to be considered when developing and prescribing treatments. Certain antipsychotic medications, especially conventional antipsychotic or neuroleptic medications, may produce symptoms of anxiety or aggravate existing anxious symptomatology in addition to causing a variety of other formidable side effects. Non-compliance rates for antipsychotic medications are high and unpleasant side effects are a major reason for this non-compliance. Alternative treatments for schizophrenia that address the issues of anxiety, reduction of unpleasant side effects, and low rates of compliance are needed.

Given the relationship between schizophrenia and anxious symptomatology and the need for new treatments to address these issues, a preclinical model of these disorders is valuable. An animal model that includes aspects of both schizophrenia and anxiety, particularly during the psychotic or break periods of the disorder when medications are most effectively used to control symptoms, could be used to study potential mechanisms and treatments for these phenomena. Although multiple models exist that study schizophrenia and anxiety independently, none has addressed the co-occurrence of these disorders and symptoms.

Animal Models in the Study of Schizophrenia

Modeling Mental Health Disorders

Animal models are frequently necessary to the study of a variety of human disorders and have been the basis for many scientific advances to understand and treat a variety of medical problems. Animal models allow for the performance of invasive and novel procedures and treatments that would be difficult or impossible to examine in a human sample.

The study of psychiatric and mental health issues frequently involves the use of animal models (Geyer & Markou, 1995; Marcotte, Pearson, & Srivastava, 2001). Such models are typically either symptomatically or mechanistically based. Symptomatic models model behaviors and attempt to mimic a specific symptom or symptoms of a disorder and find methods for treating these

symptoms. Mechanistic models are concerned with modeling potential mechanisms that explain abnormal behaviors or symptoms associated with specific disorders. Researchers are divided about which type of model is superior for studying mental health conditions, but generally agree that both have played significant roles in better understanding particular conditions.

Although many animal models may appear to lack face validity, they are based upon neuroscientific and psychological principles and evidence that mental health issues arise from a primary dysfunction of neuronal systems (Cowan, Harter, & Kandel, 2000). Various mechanistic theories and novel treatments that could not be tested in humans can be explored by modeling these dysfunctions in animals (Marcotte et al., 2001).

Models of Schizophrenia

Schizophrenia is a particularly difficult disorder to model because of its large variety of symptoms, many of which are based in cognitive domains and are difficult to recreate in animals, variable course and outcome, and possible genetic and environmental influences. As a result, current animal models of schizophrenia are designed predominantly to model specific aspects of the disorder rather than the entire human condition. Similarly, the models, unlike the disorder, are derived through a single type of manipulation: environmental, genetic, or pharmacological. Although face validity is difficult to produce in animal models of schizophrenia, many models have consistent predictive and construct validity (Marcotte et al., 2001).

There are multiple existing animal models of schizophrenia that model particular positive or negative symptoms of the disorder. Environmental models, sometimes also conceptualized as neurodevelopmental models, rely on early presentations of experiential stress, such as maternal separation (Liu et al., 1997) and early social isolation (Geyer, Wilkinson, Humby, & Robbins, 1993; Wilkinson, Killcross, Humby, Hall, Geyer, & Robbins, 1994). These stressful exposures induce sensorimotor and cognitive deficits similar to those reported in schizophrenics. Animal models also can be derived by genetic manipulations. Knockout technology in mice and selective breeding techniques in rats have resulted in strains of animals that have physiological and behavioral traits that mimic schizophrenia. Particular strains of rats (*i.e.,* apomorphine sensitive APO-SUS rats) have been selectively bred to maximize a variety of behavioral and biochemical features associated with schizophrenia (Ellenbroek, Geyer, & Cools, 1995; Paylor & Crawley, 1997). Scientists also have modeled variations in the dopaminergic, adrenergic, and glutamatergic systems and consequent behavioral disruptions by selectively suppressing expression of these classes of receptors in mice (Sibley, 1999). The neurotransmitter systems affected by schizophrenia (dopaminergic, serotonergic, and glutamatergic) also can be manipulated pharmacologically, one of the most common techniques for establishing animal models of the disorder.

Pharmacologically-induced glutamatergic models of schizophrenia are particularly relevant to the current research because the glutamate system is affected in the ketamine model that was used for this doctoral dissertation

research project. Various aspects and symptoms of schizophrenia can be induced in animals as well as humans through the administration of *N*-methyl-*D*-aspartate (NMDA) receptor antagonists. Glutamatergic disruptions have been implicated in the etiology of schizophrenia (Harrison, 1999), lending validity to glutamate-based models.

The non-competitive NMDA antagonist phencyclidine (PCP) has been used in several studies to model symptoms of psychosis in humans (Abi-Saab et al., 1998; Geyer et al., 2001; Marcotte et al., 2001). PCP administration also causes cognitive disruptions in rats that are consistent with the cognitive deficits associated with schizophrenia (Geyer et al., 2001; Marcotte et al., 2001). There are significant drawbacks to the use of PCP to model schizophrenia though, including high abuse potential and non-specific neurochemical and behavioral effects.

MK-801 (dizocilpine maleate) is another NMDA antagonist that has been used to model the cognitive deficits associated with schizophrenia in rodents. MK-801 is similar to PCP in its behavioral effects, including its ability to significantly disrupt prepulse inhibition (PPI) of the acoustic startle reflex (ASR). Prepulse inhibition (PPI) of the acoustic startle response (ASR) is the process by which a weak, non-startling acoustic stimulus presented prior to a startling acoustic stimulus inhibits the startle response. It is commonly viewed as an operational measure of sensorimotor gating and attentional processing of environmental stimuli (Braff, 1993; Braff & Geyer, 1990). Disruption of sensorimotor gating, specifically PPI, is a common cognitive deficit in

schizophrenia (Braff, Grillon, & Geyer, 1992) and is commonly used as a model of schizophrenic cognitive disruptions in rodents (Geyer et al., 2001; Swerdlow & Geyer, 1998; Weiss & Feldon, 2001). Our laboratory has used the MK-801 model to model the PPI deficits of schizophrenia for the purpose of testing novel treatment compounds (Phillips, 2003). Like PCP, there are drawbacks to the use of MK-801 to model schizophrenia, including non-specific neurochemical and behavioral effects. Neither PCP nor MK-801 is a suitable model for the current research because they also are anxiolytic, rather than anxiogenic.

Ketamine Model of Schizophrenia

Ketamine is a non-competitive NMDA antagonist and is 10 - 50 times less potent than PCP. Ketamine's decreased potency makes it a "cleaner" drug, with a more NMDA-specific profile of effects. Like PCP, subanesthetic doses of ketamine reportedly cause psychotomimetic symptoms and behavior in humans including paranoid ideation, delusions, blunted affect, psychomotor retardation, and illusions and perceptual distortions (Abi-Saab et al., 1998). Additionally, ketamine causes significant cognitive function disruptions in rats that are consistent with the cognitive deficits associated with schizophrenia. Mansbach, Carver, and Zorn (2001) and Swerdlow, Bakshi, Waikar, Taaid, and Geyer (1998) reported significant disruption of prepulse inhibition (PPI) of the acoustic startle reflex in adult male Wistar rats treated with ketamine (3.0 - 10.0 mg/kg) with prepulses ranging from 72 to 82 dB and startle stimului ranging from 118 to 120

dB. These PPI disruptions are consistent with cognitive deficits reported in schizophrenics (Braff et al., 1992).

Ketamine is unique and differs from other noncompetitive NMDA antagonists in that it is anxiogenic, or causes an increase in anxiety-like behaviors in rats. In a study of the effects of ketamine on locomotor activity, ketamine increased the amount of time spent in the perimeter and decreased time spent in the center of an open field locomotor activity chamber in adult male Long-Evans rats (Hetzler & Waulet, 1985). The open field test of locomotor activity can be used to index a variety of normal and abnormal behaviors. A rodent's natural tendency is to prefer the periphery of the arena and to spend a majority of the time moving close to the walls – a behavior known as thigmotaxis. Increased time spent in the center of the arena and increases in the ratio of center time to time in the periphery are considered to be indications of anxiolysis (Choleris, Thomas, Kavaliers, & Prato, 2001; Prut & Belzung, 2003). Additionally, acute administration of ketamine (7.0 mg/kg) to adult male Wistar rats also increased the appearance of anxiety-like behaviors in the social interaction test and on the elevated plus maze (Silvestre, Nadal, Pallares, & Ferre, 1998). The elevated plus maze (EPM) is a cross-shaped apparatus with two open and two closed arms that is raised above the floor and is one of the most commonly used measures of anxiety and anxiety-like behaviors in rodents (Belzung & Griebel, 2001; Bourin, 1997; File, 1987; Hogg, 1996; Rodgers, Cao, Dalvi, & Holmes, 1997). Percent time spent in the open arms and percentage of open arm entries are used to index anxiety (Fernandes & File, 1996; Hogg, 1996;

Rodgers & Dalvi, 1997). Social interaction tests also can be used to examine anxiety-like behaviors in rodents. As an index of anxiety, total time spent engaged in social interaction is quantified. A treatment-related increase in social interaction is indicative of anxiolysis, whereas a decrease in time spent in social interaction is considered to indicate anxiogenesis (File & Hyde, 1978; File & Seth, 2003). Ketamine may be well-suited to model symptoms of anxiety co-occuring with the cognitive symptoms of schizophrenia during psychotic or break periods of the disorder. Ketamine's disruption of cognitive functions and anxiogenic profile in rats present the unique opportunity to study symptoms of both schizophrenia and anxiety in an animal model.

Ketamine is not as well studied as PCP and MK-801 as an animal model for psychopathologies. Whereas ketamine provides a proven model for the cognitive disruptions associated with schizophrenia and is reportedly anxiogenic, ketamine has not been validated as a pharmacologically-induced model of anxiety, alone or in combination with symptoms of schizophrenia. Ketamine, being an anesthetic compound, also may have non-specific behavioral effects and a tendency, even at subanesthetic dosages, to alter mobility in rodents. The current research examined the validity of ketamine as a model of anxiety and examined ketamine-induced cognitive deficits in rats.

CONCEPTUAL RESEARCH MODEL

This doctoral dissertation research is based upon a conceptual model of the relationship between anxiety and cognitive deficits in schizophrenics that was tested in an animal model. Anxiety predisposes individuals to distractibility and makes it difficult for them to gate out irrelevant information and stimuli. Braunstein-Bercovitz and colleagues suggest that the attentional disruptions commonly displayed by individuals with schizophrenia and schizophrenia-like disorders may not be specific symptoms of the disorder, but rather may be a result of the heightened anxiety experienced by these individuals (Braunstein-Bercovitz, 2000; Braunstein-Bercovitz, et al., 2002). Braunstein-Bercovitz based this hypothesis, in part, on her factor analysis of the Schizotypal Personality Questionnaire (SPQ; Raine, 1991) and correlation of subjects' factor scores with their trait-anxiety scores as measured by the State and Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970). She reported a significant relationship between trait anxiety and cognitive deficits in high schizotypal individuals such that latent inhibition (a common measure of cognitive performance) was reduced in high-trait-anxious as compared to low-trait-anxious individuals. Additionally, a regression analysis indicated that, although both trait-anxiety scores (STAI) and schizotypal scores (SPQ) independently accounted for latent inhibition disruptions, the contributions of the STAI scores were stronger (Braunstein-Bercovitz, 2000). In addition to Braunstein-Bercovitz's findings of a relationship between anxiety and cognitive disruption in high-schizotypal

individuals, numerous studies have reported similar significant deficits in cognitive function in individuals with higher psychosis-prone scores on a variety of scales measuring psychoses (for an extensive review, please see Braunstein-Bercovitz et al., 2002). Based upon these reports and her own empirical studies, Braunstein-Bercovitz posited that the anxiety experienced by schizotypal and schizophrenic patients may be enough to induce cognitive disruptions, or it may aggravate existing cognitive deficits in these populations. To date, this interpretation had not been empirically evaluated in humans or in an animal model.

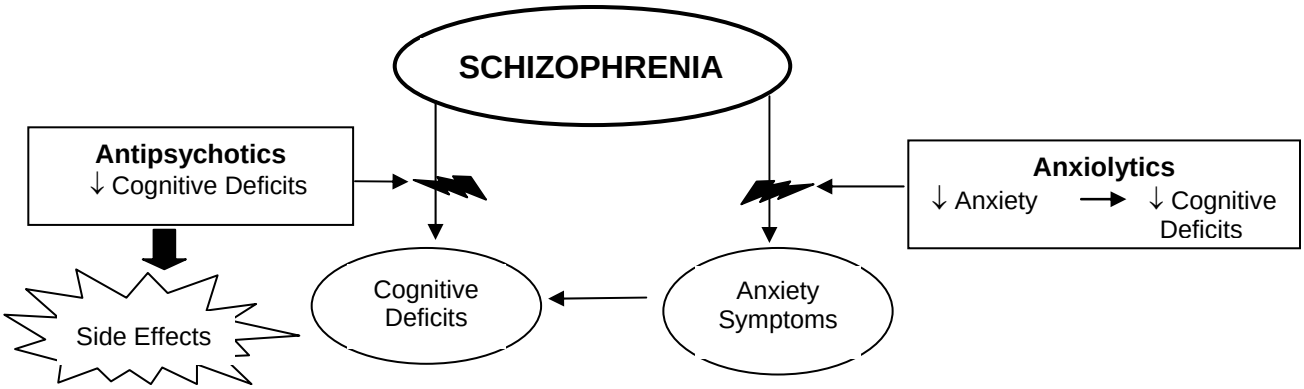


Figure 1. Proposed model of the relationship between anxiety symptoms and schizophrenia-associated cognitive deficits and their treatment with antipsychotic or anxiolytic medications

Figure 1 depicts the proposed relationship between anxiety and cognitive deficits in schizophrenia and their treatment. Cognitive deficits in schizophrenics can be decreased by antipsychotic medications. However, most antipsychotics cause numerous side effects and have a low rate of compliance. In addition, some antipsychotics, especially of the conventional or typical varieties, may cause or increase existing anxiety symptoms in schizophrenics. An alternative

treatment, as presented in this model, is to medicate the cognitive deficits **indirectly** by using anxiolytic medications that are better tolerated and cause fewer negative side effects. Based upon this model, a reduction of anxiety would indirectly minimize or alleviate the cognitive deficits as well. Treatment of schizophrenia that incorporates anxiolytic medications in place of antipsychotics, or using anxiolytics adjunctively to minimize the number and amount of antipsychotic medications needed, would potentially provide for a better quality of life for schizophrenics.

Towards the investigation of anxiolytics as a treatment for schizophrenia, the current research examined whether anxiolytics are effective, relative to antipsychotics, in reducing anxiety and cognitive deficits in the ketamine model of cognitive deficits and anxiety-like behaviors. These studies were designed to evaluate the effects of an atypical antipsychotic (clozapine) and an anxiolytic (alprazolam) in an animal model of schizophrenia and anxiety. It used an established ketamine-induced animal model of the cognitive disruptions common in schizophrenia. Additionally, pilot studies conducted in this laboratory revealed that ketamine administration also induces anxiety-like behaviors. The combined cognitive disruptions and anxiety-like behaviors model aspects of schizophrenia with concurrent anxiety symptoms. This work evaluated the effect of the antipsychotic and anxiolytic medications on cognitive deficits and anxiety-like behaviors in this ketamine-induced animal model of schizophrenia.

Antipsychotics and Anxiolytics in the Ketamine Model

Treatment of Schizophrenia with Antipsychotics

The first-line treatment for schizophrenia is generally pharmacological. The historical use of neuroleptics or conventional antipsychotics, such as haloperidol and chlorpromazine, has decreased since the advent of the newer atypical antipsychotics, such as clozapine, olanzapine, and ziprasidone. The atypical antipsychotics appear to be equally, or more, effective in treating schizophrenia, but with fewer side effects and are now considered the first-line medications for schizophrenia treatment (USDHHS, 1999).

Treatment of Schizophrenia with Anxiolytics

In an attempt to find better treatments, several other types of psychoactive drugs have been tested in schizophrenics. Attempts to treat schizophrenia with anxiolytic medications began in the 1960s. Benzodiazepines have been used with moderate success to alleviate symptoms of agitation, aggression, and catatonia in people with the disorder. Benzodiazepines cause muscle relaxation in addition to their ability to decrease anxiety. As a result of this relaxation effect, certain benzodiazepines have been used as an adjunctive treatment to antipsychotic medications to manage the common neuroleptic side effect of tardive dyskinesia. A survey of medication use in outpatient schizophrenics found that patients treated with benzodiazepines required less neuroleptic medication than those receiving neuroleptics alone (Pecknold, 1993).

The use of anxiolytics in combination with antipsychotics to treat schizophrenia has been moderately successful in reducing negative emotional behaviors and medication side effects, but the effects of these anxiolytic medications on cognitive impairments in schizophrenics had not been studied. The conceptual model that drives this research is based upon the thesis that anxiolytic medications will attenuate or alleviate the cognitive impairments as well as the anxiety-like behaviors in an animal model of these mental health conditions. With previous studies of anxiolytic medications in schizophrenia supporting a role for anxiolytics in treatment, this possibility merits research attention. Minimizing the need for the poorly-tolerated antipsychotics may help to address issues of compliance and adherence associated with schizophrenia treatment.

Antipsychotics in the Ketamine Model

Several antipsychotics, including clozapine, ziprasidone, haloperidol, and chlorpromazine, have been used to successfully restore sensorimotor gating in animals treated with ketamine. Atypical antipsychotics are more effective at lower dosages at reversing ketamine's disruptive cognitive effects. Swerdlow et al. (1998) reported that the atypical antipsychotics sertraline (Seroquel®) and clozapine (Clozaril®) both significantly attenuated ketamine-induced pre-pulse inhibition (PPI) deficits in adult male Wistar rats. Additionally, Mansbach et al. (2001) reported restoration of PPI deficits in ketamine-treated adult male Wistar rats following administration of the atypical antipsychotics clozapine (Clozaril®)

or ziprasidone (Geodon®). These investigations provide an empirical foundation and established dosages to examine effects of clozapine in the ketamine model in rats. However, the effects of these antipsychotic medications on the anxiety-like behaviors also induced by ketamine had not been studied.

Anxiolytics in the Ketamine Model

The effects of anxiolytics on sensorimotor gating in animal models have not been well studied. Specifically, there are no published studies examining the effects of these medications on disruptions of the acoustic startle reflex (ASR) and prepulse inhibition (PPI) of the ASR in any of the NMDA antagonist models, including the ketamine model. Although anxiolytics are consistently successful in reducing the occurrence of anxiety-like behaviors in multiple paradigms, their effects on ketamine-induced anxiety-like behaviors was not known.

OVERVIEW

The current research examined the effects of a standard pharmacological treatment for schizophrenia (the antipsychotic clozapine), a non-traditional pharmacological treatment (the anxiolytic alprazolam), and a combination of both treatment agents, on cognitive deficits and anxiety in a ketamine-induced rat model of schizophrenia. These studies examined the thesis that the cognitive deficits associated with schizophrenia can be medicated indirectly using anxiolytics, medications that are better tolerated than antipsychotic medications. In addition, these studies served to evaluate ketamine administration as a pharmacological model for comorbid symptoms of schizophrenia and anxiety.

Independent variables:
- Ketamine (0 and 8.5 mg/kg, administered subcutaneously)
- Clozapine (0, 3.75, and 7.5 mg/kg, administered intraperitonealy)
- Alprazolam (0, 0.75, and 1.5 mg/kg, administered intraperitonealy)
- Combination (3.75 mg/kg Clozapine and 0.75 mg/kg Alprazolam, administered intraperitonealy)

Dependent Variables:
- Acoustic Startle/Prepulse Inhibition (Cognitive)
- Passive Avoidance (Cognitive)
- Elevated Plus Maze (Anxiety)
- Open Field Locomotor Activity (Anxiety)
- Social Interaction (Anxiety)

Selection of Independent Variables

This work studied the effects of: (1) clozapine, an atypical antipsychotic, on cognitive deficits and anxiety-like behaviors in the ketamine model, (2) alprazolam, an anxiolytic, on cognitive deficits and anxiety-like behaviors in the ketamine model, and (3) a combination treatment consisting of low doses of clozapine and alprazolam on cognitive deficits and anxiety-like behaviors in the ketamine model.

Ketamine

Ketamine was selected to induce the animal model of schizophrenia based upon ketamine's ability to consistently and significantly disrupt cognitive functions in rats (sensorimotor gating and prepulse inhibition) that are consistent with the cognitive deficits associated with schizophrenia (Mansbach et al., 2001; Swerdlow et al., 1998). Ketamine also was chosen because it increases anxiety-like behaviors in rats (*i.e.,* increases time spent in the center of an open arena, decreases social interaction, decreases time spent in the open arms of the

elevated plus maze) (Hetzler & Waulet, 1985; Silvestre et al., 1998) – a property unique to ketamine relative to other NMDA antagonists. Preliminary data from our laboratory are presented below that are relevant to this research project.

Using an animal model in the current research allowed for the study of particular symptoms (information processing/cognitive deficits) and behaviors (anxiety-like behaviors). Isolating such symptoms and behaviors for study is difficult to achieve in a human population, particularly a disordered population, which has numerous pharmacological, social, and experiential influences that may interfere with these processes and behaviors. In addition, this research was designed to determine the validity of ketamine as an animal model of schizophrenia and anxiety by examining the effects of antipsychotic and anxiolytic medications on a variety of behaviors in the ketamine model. The use of the animal model in this research serves a dual purpose: validation of the model and an initial empirical study of a proposed theory.

Antipsychotic (Clozapine)

Clozapine is an atypical antipsychotic that is considered to be the prototype of the atypical antipsychotics and serves as a reference compound in the development of new, atypical antipsychotic medications (Stahl, 2002). It is a 5-HT_{2A} – D_2 antagonist with activity at a number of other sites including multiple serotonin, dopamine, and alpha adrenergic sites (Stahl, 2002). Although clozapine is associated with unusual side effects such as agranulocytosis in approximately 1% of patients treated with the drug, it has multiple benefits.

These benefits include a minimal risk of extrapyramidal side effects, reduction of suicidal behavior, and success in treating positive and negative symptoms of schizophrenia (Llorca & Pere, 2004). In addition, there are multiple reports of clozapine's efficacy in restoring cognitive deficits in animal models of schizophrenia-like sensorimotor gating deficits. Clozapine administration (3.2 mg/kg - 7.5 mg/kg) successfully restored ketamine-induced cognitive disruptions, as measured by prepulse inhibition of the acoustic startle reflex with varying prepulses and startle stimuli, in adult male Wistar and Sprague-Dawley rats (Mansbach et al., 2001; Swerdlow et al., 1998). There are conflicting reports of the effects of clozapine on measures of anxiety-like behaviors in rodents. In mice, the acute administration of low doses of clozapine (0.1 - 0.4 mg/kg) resulted in slightly less time spent in the open arms of the elevated plus maze relative to control animals, suggesting a slight anxiogenic effect (Manzaneque, Brain, & Navarro, 2002). However, a study by Cao and Rodgers (1997) reported no effects of acutely administered clozapine (0.3 - 6.0 mg/kg) on mouse behavior in the elevated plus maze. In the only published study of the effects of clozapine on a measure of anxiety in ketamine-treated rats, 5.0 mg/kg clozapine had no affect on time spent engaged in social behaviors during a test of social interaction in animals pre-treated with subchronic ketamine (30 mg/kg ketamine, administered daily for 5 days) in adult male, Sprague-Dawley rats (Becker & Grecksch, 2004). The effects of clozapine on anxiety-like behaviors in an acute ketamine model have not been studied. Clozapine was selected for this research based on its proven effectiveness in mild and treatment-resistant cases of

schizophrenia as well as its demonstrated ability to restore cognitive deficits in the ketamine model. This research project was designed to replicate the cognitive findings cited above and to determine what effects, if any, clozapine has on other cognitive measures and on anxiety-like behaviors in the ketamine model.

Anxiolytic (Alprazolam)

Alprazolam, a benzodiazepine, was selected to be tested in the ketamine model because of its frequent use in the treatment of a variety of anxiety disorders and related symptoms. Multiple reviews report alprazolam to be an effective treatment for a variety of anxiety disorders, including generalized anxiety disorder (GAD) (Rickels & Rynn, 2002; Verster & Volkerts, 2004) and panic disorder, with and without agoraphobia (Kasper & Resinger, 2001; Verster & Volkerts, 2004), as well as non-anxiety disorders such as depression (Verster & Volkerts, 2004). Alprazolam has a fast onset of action with noticeable symptom relief occurring almost immediately, and no tolerance to its therapeutic effects. These characteristics were desirable for the current research, a repeated acute administration design. Additionally, alprazolam has been used in combination with neuroleptics in schizophrenic patients with positive results (Pecknold, 1993). In rat and mouse models, acute administration of alprazolam consistently reduces the appearance of common anxiety-like behaviors on a variety of measures, including the elevated plus maze (File & Pellow, 1985; Griebel, Sanger, & Perrault, 1996; Hascoet & Bourin, 1998; Martin, Ballard, &

Higgins, 2002; Prunell, Escorihuela, Fernandez-Teruel, Nunez, & Tobena, 1994). Although there is a report of a benzodiazepine attenuating ketamine-induced hyperlocomotion in mice (Irifune et al., 1998), there are no published reports of the effects of any benzodiazepines, including alprazolam, on ketamine-induced anxiety-like behaviors in rodents. Alprazolam's ability to affect ketamine-induced cognitive disruptions is not known. Alprazolam was selected for the current research based on its quick onset, lack of reported tolerance development, effectiveness in treating a variety of anxiety-related disorders, and its effectiveness in a variety of animal models of anxiety-like behaviors. This research project was designed to determine the effects of alprazolam on sensorimotor gating and other cognitive deficits and on anxiety-like behaviors in the ketamine model.

Combination Treatment

A combination treatment was included to determine the effects of concurrent administration of an antipsychotic and an anxiolytic in the ketamine model. A survey of medication use in outpatient schizophrenics found that patients treated with anxiolytics required less neuroleptic medication than those receiving neuroleptics alone (Pecknold, 1993). These findings suggest a benefit of concurrent administration of anxiolytics and antipsychotics, although the effects of this combination on cognitive deficits and anxiety are not known. This research project was designed to determine the effects of concurrent

administration of alprazolam and clozapine on sensorimotor gating and other cognitive deficits and on anxiety-like behaviors in the ketamine model.

Selection of Dependent Variables: Cognitive Measures

Cognitive disruptions and dysfunctions are common in schizophrenia and other psychotic disorders. Executive function, verbal and visuospatial working memory, other types of memory, learning processes and attention are all compromised in patients suffering from schizophrenia and other disorders of the psychotic spectrum (Sharma & Antonova, 2003).

Prepulse Inhibition

Prepulse inhibition (PPI) of the acoustic startle response (ASR) is the process by which a weak, non-startling acoustic stimulus presented prior to a startling acoustic stimulus inhibits the startle response. It is commonly viewed as an operational measure of sensorimotor gating and attentional processing of environmental stimuli (Braff, 1993; Braff & Geyer, 1990). Disruption of sensorimotor gating, specifically PPI, is a common cognitive deficit in schizophrenia (Braff, Grillon, & Geyer, 1992) and is commonly used as a model of schizophrenic cognitive disruptions in rodents (as reviewed in Geyer et al., 2001; Swerdlow & Geyer, 1998; Weiss & Feldon, 2001).

Despite isolated reports that ketamine may increase PPI in normal human subjects, multiple human studies and the entire animal literature suggest that ketamine consistently decreases PPI (Braff, 1993). Multiple studies report that ketamine (at dosages of 5.0 - 10.0 mg/kg) significantly decreases prepulse

inhibition using a range of preulse and startle stimuli in adult male rats in a manner similar to that in schizophrenics (Brooks & Mansbach, 1997; DeBruin, Ellenbroek, Cools, Coenen, & Van Luijtelaar, 1999; Johansson, Jackson, Zhang, & Svensson, 1995; Mansbach & Geyer, 1991; Swerdlow, Caine, Braff, & Geyer, 1992).

Our laboratory has extensive experience with ASR and PPI (*e.g.,* Acri, 1994; Acri, Brown, Saah, & Grunberg, 1995; Acri, Morse, Popke, & Grunberg, 1994; Faraday & Grunberg, 2000; Faraday, Rahman, Scheufele, & Grunberg, 1998; Phillips, 2003; Phillips & Grunberg, 2003; Popke, Tizabi, Rahman, Nespor, & Grunberg, 1997). In pilot studies conducted in our laboratory, ketamine at a dosage of 8.5 mg/kg (administered acutely, subcutaneously) disrupted sensorimotor gating and significantly decreased ASR and PPI in adult male Wistar rats (please see Preliminary Findings).

Passive Avoidance

Passive avoidance is an example of a fear motivated avoidance test in which the animal must refrain from executing a previous response to avoid punishment – typically a low-voltage shock. Performance deficits in this measure may reflect interference with the ability to preserve information (Myhrer, 2003) and may index working memory and/or the ability to process information from short-term to long-term memory. Disruption of working memory is a common cognitive deficit in patients with schizophrenia (Fleming et al., 1997;

Gold, Carpenter, Randolph, Goldberg, & Weinberger, 1997; Keefe, Lees-Roitman, & Dupre, 1997; Sullivan, Shear, Zipursky, Sagar, & Pfefferbaum, 1997).

It has consistently been reported that ketamine disrupts passive avoidance learning in rodent models when administered during the acquisition phase of the task. In mice, ketamine dose-dependently inhibits the acquisition of memory (Saha, Chugh, Sankaranarayana, & Datta, 1990; Uchihashi, Kuribara, Isa, Morita, & Sato, 1994). Similarly, ketamine disrupts acquisition of the passive avoidance fear-response in rats. Adult male Wistar rats displayed significantly impaired learning acquisition and increased latencies after a 24 hour waiting period on the passive avoidance task following administration of 10.0 - 12.5 mg/kg ketamine on the training day (Babar, Ozgunen, Melikov, & Gaibova, 1998; Gandolfi, Dall'Olio, Roncada, & Montanaro, 1990; Jones, Bauerle, & DeNoble, 1990). Whether antipsychotic and anxiolytic medications restore deficits in passive avoidance is not known. Our laboratory has used the passive avoidance measure to study the effects of drugs on working memory in rats (Popke et al., 1997; Skvorc, Davis, Morse, & Grunberg, 1996).

Selection of Dependent Variables: Anxiety Measures

Anxiety symptomatology and phobias commonly occur in conjunction with schizophrenia (APA, 2000) and further impair schizophrenics' functioning and treatment compliance. A variety of measures have been proposed as models of anxiety and anxiety-like behaviors in rodents. (For extensive reviews of behavioral models of anxiety in animals, please see Belzung & Griebel, 2001;

Bourin, 1997; File, 1987; Rodgers, Cao, Dalvi, & Homes, 1997.) The most widely used measures include: elevated plus maze behavior, open field locomotor activity, and social interaction. Our laboratory has experience with all of these measures (Cook, 2001; Elliott, Faraday, Phillips, & Grunberg, 2004; Faraday, Elliott, Phillips, & Grunberg, 2003; Morse et al., 1997; Scheufele, Faraday, & Grunberg, 2000). These measures index aspects of anxiety and anxiety-like behaviors in rodents based upon uncertainty generated by placing rodents in an unfamiliar environment (File, 1987).

Elevated Plus Maze

The elevated plus maze (EPM) is a cross-shaped apparatus with two open and two closed arms that is raised above the floor. The EPM is one of the most commonly used measures of anxiety and anxiety-like behaviors in rodents (Belzung & Griebel, 2001; Bourin, 1997; File, 1987; Hogg, 1996; Rodgers et al., 1997). Percent time spent in the open arms and percentage of open arm entries are used to index anxiety (Fernandes & File, 1996; Hogg, 1996; Rodgers & Dalvi, 1997). These parameters have been validated both pharmacologically and behaviorally. Specifically, anxiogenic drugs decrease these parameters, anxiolytic drugs increase these parameters, and animals confined to the open arms exhibit more fear responses than animals confined to closed arms (Fernandes & File, 1996; Pellow, Chopin, File, & Benley, 1985; Rodgers & Dalvi, 1997). Our laboratory has used the elevated plus maze to study the effects of

nicotine and other drugs on anxiety-like behaviors in rats (Cook, 2001; Elliott, et al., 2004).

Ketamine (7.0 mg/kg), acutely administered to adult male Wistar rats, has an anxiogenic effect on elevated plus maze behavior, decreasing entries into and time spent in the open arms (Silvestre et al., 1998). In pilot studies in our laboratory, ketamine, administered via subcutaneous injection at a dosage of 8.5 mg/kg, also significantly decreased entries into the open arms and time spent in the open arms in adult male Wistar rats (please see **Preliminary Findings**).

Open Field Locomotor Activity

The open field test of locomotor activity can be used to index a variety of normal and abnormal behaviors and is one of the most frequently used measures in our laboratory. Of interest to the study of anxiety is a rodent's natural tendency to prefer the periphery of the arena and to spend a majority of the time ambulating close to the walls – a behavior known as thigmotaxis. Increased time spent in the center of the arena as well as increases in the ratio of center time to time in the periphery are considered indications of anxiolysis (Choleris, Thomas, Kavaliers, & Prato, 2001; Prut & Belzung, 2003). Tests of general locomotor activity are common in the study of drug effects and are used frequently in our laboratory. As an index of anxiety, locomotor activity has been used in our laboratory to study the effects of nicotine as well as other drugs on anxiety-like behaviors in rats (Cook, 2001; Elliott, et al., 2004).

High acute dosages of ketamine (50 mg/kg) produce an increase in locomotion in adult male Long-Evans rats that consists mainly of ambulation around the perimeter of the open field (Hetzler & Waulet, 1985). Increased time spent in the perimeter and decreased time spent in the center of an open arena is indicative of anxiogenesis. Pilot studies in our laboratory, using an acute dosage of 8.5 mg/kg ketamine, found that ketamine significantly decreased time spent in the center of the arena with little or no effect on general locomotion in adult male Wistar rats (please see **Preliminary Findings**).

Social Interaction

Social interaction tests also can be used to examine normal and abnormal rodent behaviors. As an index of anxiety, total time spent engaged in social interaction is quantified. A treatment-related increase in social interaction is indicative of anxiolysis, whereas a decrease in time spent in social interaction is believed to indicate anxiogenesis (File & Hyde, 1978; File & Seth, 2003). Our laboratory has used this test to examine the effects of nicotine and housing condition on social interaction in rats (Scheufele, Faraday, & Grunberg, 2000). Work by Silvestre and colleagues (1998) has demonstrated that acute administration of 7 mg/kg ketamine has an anxiogenic effect on social interaction, decreasing time spent in social behaviors as well as central activity in the social arena in adult male Wistar rats.

Preliminary Findings

Preliminary studies were conducted that incorporate several of the independent and dependent variables proposed in this study. These studies were performed to verify the ability of this laboratory to produce the ketamine-induced cognitive disruptions and anxiety-like behaviors that are required to establish the model for this doctoral research project.

Preliminary study #1 examined the effects of acutely administered ketamine (0 or 8.5 mg/kg) on ASR/PPI in 24 adult, male Wistar rats of the same approximate age as the subjects in the proposed research. This ketamine dosage was chosen based upon reports of the effects of ketamine on measures of cognition (Mansbach & Geyer, 1991; Swerdlow et al., 1998). Ketamine had no significant effect on the acoustic startle response (ASR). Ketamine significantly decreased prepulse inhibition (PPI) relative to saline controls [$F(4, 19) = 4.61$, $p<.01$]. This decrease in % PPI was consistent with previous research using ketamine and with accepted models of cognitive disruptions in schizophrenia.

Preliminary study #2 examined the effects of acutely administered ketamine (0 or 8.5 mg/kg) on elevated plus maze performance (EPM) and center time in 24 adult, male Wistar rats of the same approximate age as the subjects in the proposed research. Ketamine-treated rats spent less time in the open arms of the elevated plus maze than did the saline controls, a difference that approached statistical significance [$F(1, 15) = 3.929$, $p = 0.066$]. Rats receiving

ketamine spent significantly less time in the center of the locomotor activity arena, relative to saline controls [$F(1, 22) = 11.20$, $p < 0.01$]. Ketamine-treated rats also spent significantly less time engaged in vertical activity [$F(1, 22) = 18.98$, $p < 0.01$]. Ketamine-treated animals also spent less time in the light portion of the light-dark apparatus than did saline controls [$F(1, 20) = 11.03$, $p < 0.01$]. These behaviors were all consistent with an anxiogenic effect of ketamine. Animals receiving ketamine did not differ significantly from those receiving saline on measures of general horizontal activity, suggesting that the findings that ketamine-treated animals spend less time in the open arms of the EPM, less time in the center of the locomotor arena, and less time in the lighted portion of the light-dark apparatus cannot be attributed to general activity differences between the groups.

The results of the preliminary studies: (1) established the appropriate ketamine dosage to produce consistent cognitive disruptions and anxiety-like behaviors, and (2) successfully measured ketamine-induced cognitive disruptions and anxiety-like behaviors using several of the proposed dependent variables. The findings of these preliminary studies supported the use of ketamine as an appropriate model to study schizophrenia-like cognitive disruptions and anxiety-like behaviors concurrently and that the proposed dependent variables were feasible and in place.

HYPOTHESES

This doctoral dissertation project is an animal model of the effects of an antipsychotic drug and an anxiolytic drug on cognitive performance and anxiety-like behaviors in a ketamine-induced animal model of schizophrenia and anxiety. Experiment #1 was conducted as a 2 (Ketamine: 0 or 8.5 mg/kg) x 6 (Drug: Vehicle Control; Clozapine [3.75, or 7.5 mg/kg], Alprazolam [0.75 or 1.5 mg/kg]; Combination [3.75 mg/kg Clozapine and 0.75 mg/kg Alprazolam]) full factorial experiment. Experiment #2 was conducted as a 2 (Ketamine: 0 or 8.5 mg/kg) x 6 (Drug: Vehicle Control; Clozapine [3.75, or 7.5 mg/kg]; Alprazolam [0.75 or 1.5 mg/kg]; Combination [3.75 mg/kg Clozapine and 0.75 mg/kg Alprazolam]) full factorial experiment.

There were four major hypotheses for Experiment #1 and four major hypotheses for Experiment #2. Cognitive measures refer to prepulse inhibition of the acoustic startle (ASR/PPI) and passive avoidance. Anxiety measures refer to the elevated plus maze (EPM), locomotor activity (specifically, time spent in the center of the arena), and social interaction.

Hypotheses Experiment #1

Hypothesis 1

Ketamine administration will cause cognitive disruptions (*i.e.,* decrease prepulse inhibition of the acoustic startle reflex and decrease learning in passive avoidance).

Rationale: Numerous studies have reported that ketamine, in dosages similar to that being used in this research project, significantly decreases prepulse inhibition in rats (Brooks & Mansbach, 1997; DeBruin et al., 1999; Johansson et al., 1995; Mansbach & Geyer, 1991; Phillips & Grunberg, 2005; Swerdlow et al., 1992). Findings from preliminary studies of the effects of ketamine (8.5 mg/kg) on ASR/PPI in this laboratory have replicated the findings of decreased % PPI following ketamine administration (please see **Preliminary Findings**). Ketamine also consistently disrupts passive avoidance learning in rats when administered during the acquisition phase of the task (Babar et al., 1998; Gadolfini et al., 1990; Jones et al., 1990).

Hypothesis 2

Clozapine will attenuate the cognitive disruptions caused by ketamine (*i.e.,* restore prepulse inhibition of the acoustic startle reflex, restore learning in passive avoidance performance).

Rationale: There are multiple reports of clozapine successfully restoring ketamine-induced disruptions of prepulse inhibition. In a study by Mansbach and colleagues (2001), the disruptive effects of 10 mg/kg ketamine on PPI was

diminished by pretreatment with 3.2 and 5.6 mg/kg clozapine. A similar restoration of decreased PPI was reported in rats receiving 6.0 mg/kg of ketamine and 7.5 mg/kg of clozapine (Swerdlow et al., 1998).

Hypothesis 3

Alprazolam will attenuate the cognitive disruptions caused by ketamine (*i.e.,* restore prepulse inhibition of the acoustic startle reflex, restore learning in passive avoidance performance).

Rationale: Based upon the hypothesis that the cognitive deficits in schizophrenia are caused or mediated by inherent anxiety (Braunstein-Bercovitz, 2000; Braunstein-Bercovitz et al., 2002), a reduction in anxiety by the anxiolytic drug alprazolam will attenuate the cognitive disruptions caused by ketamine.

Hypothesis 4

The combination treatment will attenuate the cognitive disruptions caused by ketamine (*i.e.,* restore prepulse inhibition of the acoustic startle reflex, restore learning in passive avoidance performance).

Rationale: Based upon the multiple reports of clozapine successfully restoring ketamine-induced disruptions of prepulse inhibition (Mansbach et al., 2001; Swerdlow et al., 1998) and the hypothesis that the cognitive deficits in schizophrenia are caused or mediated by inherent anxiety (Braunstein-Bercovitz, 2000; Braunstein-Bercovitz et al., 2002), concurrent administration of both medications will attenuate the cognitive disruptions caused by ketamine.

Hypotheses Experiment #2

Hypothesis 1

Ketamine administration will increase anxiety-like behaviors (*i.e.,* decrease time spent in the open arms of the elevated plus maze, decrease time spent in the center of the locomotor arena, and decrease time spent in social interaction).

Rationale: Hetzler and Waulet (1985) reported that ketamine increased time spent in the periphery of a locomotor chamber and decreased time spent in the center – behaviors indicative of anxiogenesis. Silvestre and colleagues (1998) reported that 7 mg/kg of ketamine decreased entries into and time spent in the open arms of the elevated plus maze (EPM). They also reported decreased time spent in active social interaction and decreased time spent in the center of the interaction arena (Silvestre et al., 1998), also suggestive of an anxiogenic compound. Findings from preliminary studies of the effects of ketamine (8.5 mg/kg) on measures of anxiety-like behaviors in this laboratory have replicated these findings of anxiogenesis following ketamine administration on the EPM, locomotor activity test, and light-dark paradigm (please see **Preliminary Findings**).

Hypothesis 2

Clozapine will have no effect on, or will increase, the anxiety-like behaviors caused by ketamine (*i.e.,* no effect to restore time spent in the open arms of the elevated plus maze, no effect to restore time spent in the center of the locomotor arena, and no effect to restore time spent in social interaction).

Rationale: Reports of the effects of clozapine on measures of anxiety-like behaviors in rodents have been mixed. Some studies report no effects of clozapine on measures of anxiety, such as the elevated plus maze and social interaction test (Cao & Rodgers, 1997; Millan, Brocco, Gobert, Schreiber, & Dekeyne, 1999). In contrast, several studies have reported anxiogenic effects of clozapine (a tendency to increase anxiety-like behaviors) on these same measures (Manzaneque, Brain, & Navarro, 2002; Rademacher, Schuyler, Kruschel, & Steinpreis, 2002).

Hypothesis 3

Alprazolam will attenuate the increase in anxiety-like behaviors caused by ketamine (*i.e.,* restore time spent in the open arms of the elevated plus maze, restore time spent in the center of the locomotor arena, and restore time spent in social interaction).

Rationale: Several studies have reported that alprazolam reduces the appearance of common anxiety-like behaviors in multiple measures of anxiety in animals. Alprazolam (0.5 - 2.0 mg/kg) increased time spent in the light portion of the light/dark apparatus (Hascoet & Bourin, 1998). In the elevated plus maze, alprazolam (dosages from 0.1 - 3.0 mg/kg) increased the percentage of open arm entries and time spent in the open arms (File & Pellow, 1985; Griebel et al., 1996; Martin et al., 2002; Prunell et al., 1994).

Hypothesis 4

The combination treatment will attenuate the increase in anxiety-like behaviors caused by ketamine (*i.e.,* restore time spent in the open arms of the elevated plus maze, restore time spent in the center of the locomotor arena, and restore time spent in social interaction).

Rationale: There are numerous reports of alprazolam decreasing anxiety-like behaviors on multiple measures of anxiety in rats (File & Pellow, 1985; Griebel et al., 1996; Hascoet & Bourin, 1998; Martin et al., 2002; Prunell et al., 1994). Although reports of the effects of clozapine on anxiety-like measures are mixed (Cao & Rodgers, 1997; Manzaneque, Brain, & Navarro, 2002; Millan, Brocco, Gobert, Schreiber, & Dekeyne, 1999; Rademacher, Schuyler, Kruschel, & Steinpreis, 2002), the anxiolytic effects of alprazolam will result in an overall attenuation of the anxiety-like behaviors caused by ketamine.

METHODS

Overview (Experiments #1 & 2)

This research was composed of two, 2 (ketamine: 0 or 8.5 mg/kg) x 6 (drug: vehicle control, clozapine [3.75 mg/kg or 7.5 mg/kg], alprazolam [0.75 mg/kg or 1.5 mg/kg], or a combination treatment [3.75 mg/kg clozapine + 0.75 mg/kg alprazolam]) full factorial design experiments with 12 rats per treatment cell. Each experiment had N = 144 rats and the entire study included 288 rats. Please see **Table 2** for a description of treatments and cell sizes for each experiment.

Table 2. Experimental Design

	Ketamine (mg/kg)	
Drugs (mg/kg)	0	8.5
Vehicle Control	n = 12	n = 12
Clozapine (3.75)	n = 12	n = 12
Clozapine (7.5)	n = 12	n = 12
Alprazolam (0.75)	n = 12	n = 12
Alprazolam (1.5)	n = 12	n = 12
Combination	n = 12	n = 12
	Total N = 144	

The current research was accomplished in two separate experiments that were not contingent on one another:

- Experiment #1 examined the effects of clozapine, alprazolam, and a combination treatment on ketamine-induced cognitive disruptions in prepulse inhibition and passive avoidance.
- Experiment #2 examined the effects of clozapine, alprazolam, and a combination treatment on ketamine-induced anxiety-like behaviors on the elevated plus maze, open field test, and social interaction test. (Please see **Table 2** for a breakdown of treatments and cell sizes for each experiment.)

Each experiment was run in four cohorts of 36 rats with all experimental conditions equally represented. Each cohort was divided into two groups (18 rats) with the groups staggered by one day to make running all animals on all measures feasible. A drug wash-out period of at least 2 days was allowed between each behavioral measure. One cohort arrived approximately every 4 weeks with a total behavioral testing time of four months, from start to finish.

The Research Design and Methodology Relevant to Experiment #1 is presented below, followed by the Results and Discussion sections for Experiment #1. The Research Design and Methodology Relevant to Experiment #2 is then presented, followed by the Results and Discussion sections for Experiment #2.

Research Design and Methods Relevant to Experiment #1

Subjects and Housing

Subjects were 144 young adult male Wistar rats, approximately 50 days old and 275 g at the start of the experiment (Charles River Laboratories). Wistar rats were chosen based on multiple reports of the significant behavioral effects of ketamine on the measures used in these experiments, including ASR and PPI (DeBruin et al., 1999; Mansbach, Carver, & Zorn, 2001), passive avoidance (Babar et al., 1998), and multiple measures of anxiety (Silvestre et al., 1997). Young adult rats, approximately 10 days after adolescence (Spear, 2000), were used to closely model the human schizophrenic condition, which most commonly manifests during young adulthood (early to late 20s) (APA, 2000; United States Department of Health and Human Services, 1999). All animals were single-housed in standard rat cages (42.5 x 20.5 x 20 cm) on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Animals were single-housed to maintain consistency between Experiment #1 and Experiment #2. Animals in Experiment #2 were single-housed to prevent interference in the social interaction measure. The housing room was maintained at room temperature (65 - 78° F) with a humidity of approximately 50% and a 12- hour reverse light cycle (lights on at 1900 and off at 0700 hours). Behavioral procedures were conducted during the dark portion of the light cycle, between 0800 and 1500 hours. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee (IACUC)

and as conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Pub, 82-23, rev. 1985).

Drugs

Chemical adjustments necessary to neutralize the pH of the various drug solutions to match that of saline were made using sodium hydroxide (NaOH). Ketamine HCl (100 mg/mL; Sigma, USA) was diluted with physiological saline and prepared in dosages of 0 and 8.5 mg/kg. These dosages were based upon pilot studies of the effects of ketamine on measures of cognition and anxiety conducted in our laboratory and other studies in the literature (Mansbach & Geyer, 1991; Swerdlow et al., 1998).

Clozapine (Sigma, USA) was dissolved in a minimal amount of 2.0 N HCl and diluted to full volume with physiological saline and prepared in dosages of 3.75 and 7.5 mg/kg. These clozapine dosages were based upon previous reports of dosages necessary to attenuate the prepulse deficits caused by ketamine administration (Swerdlow, Bakshi, & Geyer, 1996; Swerdlow et al., 1998).

Alprazolam (Sigma, USA) was dissolved in a minimal amount of 2.0 N HCl and diluted to full volume with physiological saline and prepared in dosages of 0.75 and 1.5 mg/kg. These alprazolam dosages were based upon previous reports of dosages necessary to attenuate anxiety-like behaviors or to induce anxiolytic behaviors (File & Pellow, 1985; Hascoet, Bourin, Colobel, Fiocco, & Baker, 2000; Prunell et al., 1994).

The combination treatment consisted of clozapine (3.75 mg/kg) and alprazolam (0.75 mg/kg), prepared separately as described above before being mixed and administered via a single injection. These dosages were chosen based on preliminary behavioral results that revealed that the low dosages of clozapine and alprazolam had the greatest behavioral effects with the fewest negative side effects (please see Results Section).

The vehicle control, clozapine, alprazolam, or combination of clozapine and alprazolam was administered intraperitonealy (ip). Ten minutes after the ip injection, animals received a subcutaneous injection of saline or ketamine. Behavioral testing began 10 minutes after saline or ketamine injections for a total of 20 minutes between treatment with the vehicle control, clozapine, alprazolam, or combination of clozapine and alprazolam, and behavioral testing.

Procedure

The data for Experiment #1 were collected over approximately 2 months (Please see **Table 3** for a detailed timeline of Experiment #1). Subjects were run in balanced groups of 18 to allow for sufficient time to complete each behavioral measure during the dark, or active, period of the subjects' light cycle. All animals underwent

Table 3. Experiment #1: Experimental Timeline

Day	**Group A**	**Group B**
1	Rats Arrive (n=18)	Rats Arrive (n=18)
2	Gentle	
3	Gentle	Gentle
4	ASR/PPI Acclim	Gentle
5		ASR/PPI Acclim
6	ASR/PPI BL	
7		ASR/PPI BL
8	ASR/PPI Test	
9		ASR/PPI Test
10		
11		
12	PA - Training	
13	PA - Test	PA - Training
14		PA - Test

ASR/PPI = Acoustic Startle and Prepulse Inhibition
PA = Passive Avoidance

acclimation sessions in the acoustic startle apparatus to reduce the contamination of responses by the stressful effects of exposure to a novel situation (Acri, 1994). On day 8 or day 9, the rats were tested in the acoustic startle apparatus following treatment with vehicle control, clozapine, alprazolam, or combination treatment, and saline or ketamine. The rats were trained and tested in the passive avoidance apparatus following treatment with a vehicle control, clozapine, alprazolam, or combination treatment, and saline or ketamine on days 12-14.

% Prepulse Inhibition: ASR amplitudes and PPI were measured in an Acoustic Response Test System (MED-ASR-310; Med Associates, Georgia, VT). Responses were measured on day 8 or 9 (Please see **Table 3** for a detailed timeline of Experiment #1). Test sessions were approximately 15 minutes and included an acclimation period followed by two trial types: startle stimulus only (20 msec white noise bursts at 110 or 120 dB) or startle stimulus + pre-pulse (110 or 120 dB preceded 100 msec by a 75 or 82 dB pure tone pre-pulse). Each trial type was presented eight times in a random order with a variable intertrial interval (mean of 15 sec). Our laboratory has regularly used these procedures to examine the effects of various drugs and stress on ASR/PPI (Acri, 1994; Acri et al., 1995; Faraday & Grunberg, 2000; Faraday et al., 1998; Popke et al., 1997).

Passive Avoidance: Passive avoidance training and testing occurred on days 12 and 13 or on days 13 and 14. Passive avoidance was measured

utilizing an automated avoidance training system (Gemini, San Diego Instruments, San Diego, CA) with scrambled, constant current shocks (0.4 mA for 1 sec). Testing took place 24 hours post-training. Latency to cross to the dark chamber was recorded on the testing day with no shock administered. This procedure was based on previous work in this laboratory (Popke et al., 1997; Skvorc et al., 1996).

Statistical Issues

Data Analyses: For all analyses, exploratory analyses were used to determine if the behavioral measures were independent. Prepulse inhibition was analyzed with a multivariate analysis of variance that included all six treatment groups because the PPI responses to different stimulus pairs were correlated. ANOVAs also were performed on the experimental groups treated with ketamine and on the groups treated with saline. Passive avoidance was analyzed using non-parametric Wilcoxon Signed-Rank tests and Kruskal-Wallis ANOVAs because the data did not meet the criteria for parametric analysis. *Post hoc* Dunnett's t tests were run when appropriate. In addition, planned comparisons (t-tests) were made between animals treated with saline and animals treated with ketamine, based on Hypothesis #1 for each experiment. These planned comparisons were restricted to the experimental groups receiving ketamine or saline alone (*i.e.,* without clozapine or alprazolam) to clearly determine whether ketamine disrupted the behavior that was measured. These analyses complemented the statistical analyses that included all treatment groups. All

tests were two-tailed and an α level of 0.05 was used to determine statistical significance.

Sample Size: The sample size (cell size of n = 12) was determined in two ways: (1) using an approximation of the n used in published reports using the same behavioral measures and the same drugs or types of drugs (Geyer et al., 2001; Silvestre et al., 1998; Swerdlow et al., 1998), and (2) a power analysis based on pilot work performed in preparation for this research.

The power analyses were conducted following the procedures of Keppel (1991), Keppel, Saufley, and Tokunga (1992), and Cohen (1988). Using the effect sizes (omega squared) obtained in preliminary studies with the same behavioral measures and drugs used in the current research, calculations to achieve a power of 0.80, as recommended by Cohen (1988), revealed that the number of animals necessary was 12 per treatment cell. These empirically-based and calculated sample sizes also are consistent with studies that we have conducted with other medications and their effects on the behavioral measures used in these experiments (Acri et al., 1995; Cook, 2001; Elliott et al., 2004; Faraday et al., 2003).

RESULTS – EXPERIMENT #1

Prepulse Inhibition

Prepulse inhibition (PPI) of the acoustic startle response (ASR) is the process by which a weak, non-startling acoustic stimulus presented prior to a startling acoustic stimulus inhibits the startle response. It is commonly viewed as an operational measure of sensorimotor gating and attentional processing of environmental stimuli (Braff, 1993; Braff & Geyer, 1990). Disruption of sensorimotor gating, specifically PPI, is a common cognitive deficit in schizophrenia (Braff, Grillon, & Geyer, 1992) and is commonly used as a model of schizophrenia-like cognitive disruptions in rodents (as reviewed in Geyer et al., 2001; Swerdlow & Geyer, 1998; Weiss & Feldon, 2001). In the present experiment, this measure was used to model schizophrenia-like cognitive disruptions and to examine the effects of clozapine, alprazolam, and a combination treatment in this ketamine-induced model of schizophrenia. Percent PPI (% PPI) was calculated using the following formula: [(Startle amplitude alone – Startle amplitude with prepulse)/Startle amplitude alone] x 100.

Planned comparisons (t-tests) were made between animals treated with saline and the drug vehicle and animals treated with ketamine and the drug vehicle (based on Hypothesis #1 for this experiment). A multivariate analyses of variance (MANOVA) was used to analyze % PPI data (percentage of prepulse inhibition to a 110 dB and to a 120 dB startle stimulus when each was preceded by a 68 or 82 dB prepulse). Individual analyses of variance (ANOVAs) were

used to determine the contributions of the individual variables (Ketamine and drug treatment). Dunnett's t-test *post hoc* analyses were performed to compare individual treatment groups to the control groups.

All reported effects are significant at $p < 0.05$ unless otherwise noted. Results are reported and graphed for % PPI to each startle stimulus (110 dB and 120 dB) when preceded by a prepulse (68 dB or 82 dB). F values, degrees of freedom, and p values for each test are reported in Table 5 in Appendix A.

Figures 2-9 present the % PPI responses for each of the prepulse (68 and 82 dB) and startle (110 and 120 dB) pairs. The top panel of each figure presents the responses of the saline-treated animals for each of the drug treatment groups. The bottom panel of each figure presents the responses of the ketamine-treated animals for each of the drug treatment groups.

Planned comparison t-tests comparing animals treated with saline to animals treated with ketamine were performed for each of the prepulse and startle stimulus pairs. Ketamine significantly decreased % PPI for both 110 dB startle stimulus trials [68 dB prepulse: $t(22)=4.86$; 82 dB prepulse: $t(22)=4.92$] and 120 dB startle stimulus trials [68 dB prepulse: $t(22)=3.72$; 82 dB prepulse: $t(22)=5.41$].

When all animals were considered together, there was a significant overall effect of ketamine [$F(4, 129)=19.03$]. Ketamine decreased % PPI to the 110 dB startle stimulus [68 dB prepulse: $F(1, 132)=47.27$; 82 dB prepulse: $F(1, 132)=27.28$] and to the 120 dB startle stimulus [68 dB prepulse: $F(1, 132)=45.41$;

82 dB prepulse: F(1, 132)=60.46]. These effects are consistent with the hypothesis that ketamine would disrupt cognitive functioning.

Individual ANOVAs were performed for each of the stimulus combinations for animals in the ketamine group to determine whether each drug attenuated the % PPI deficits caused by ketamine. There was a significant drug effect for a startle stimulus of 120 dB with a prepulse of 82 dB [F(5, 66)=3.97] and a trend for a significant drug effect for a startle stimulus of 120 dB with a 68 dB prepulse [(F(5, 66)=2.03), p= 0.086]. *Post hoc* analyses of these two stimulus pairs were performed using Dunnett's t tests. There were no significant drug group differences for the 68 dB prepulse and the 120 dB stimulus trials. In trials utilizing the 82 dB prepulse and 120 dB stimulus pairing, the low dose of clozapine (3.75 mg/kg) significantly increased % PPI relative to animals treated with ketamine. Neither dose of alprazolam (0.75 or 1.5 mg/kg) altered the % PPI deficits caused by ketamine. In addition, the combination of 3.75 mg/kg clozapine and 0.75 mg/kg alprazolam had no significant effect on % PPI deficits caused by ketamine.

PPI Summary: As hypothesized, ketamine significantly decreased % PPI relative to saline-treated animals, consistent with ketamine causing cognitive disruption. Clozapine (3.75 mg/kg) significantly attenuated this ketamine-induced PPI disruption in trials using 82 dB prepulse and 120 dB startle stimuli. Neither alprazolam nor the combination of clozapine + alprazolam attenuated the ketamine-induced PPI disruption.

Figure 2. Saline

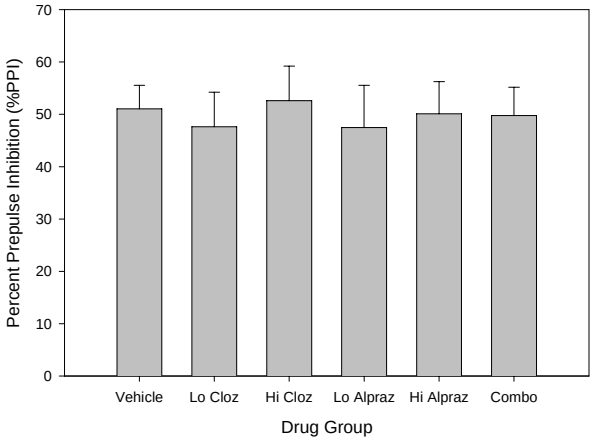
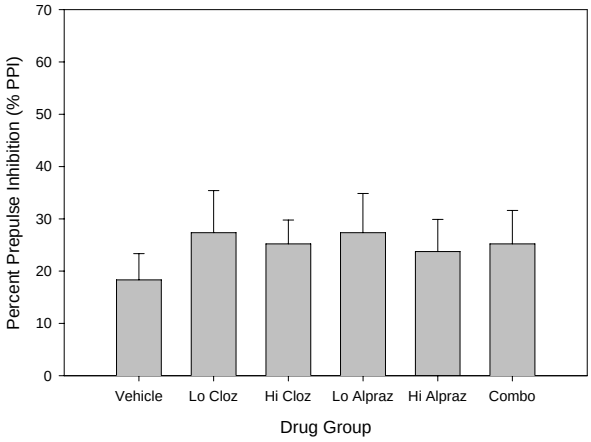


Figure 3. Ketamine



Figures 2-3. Percent prepulse inhibition to a prepulse of 68 dB and a startle stimulus of 110 dB in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 4. Saline

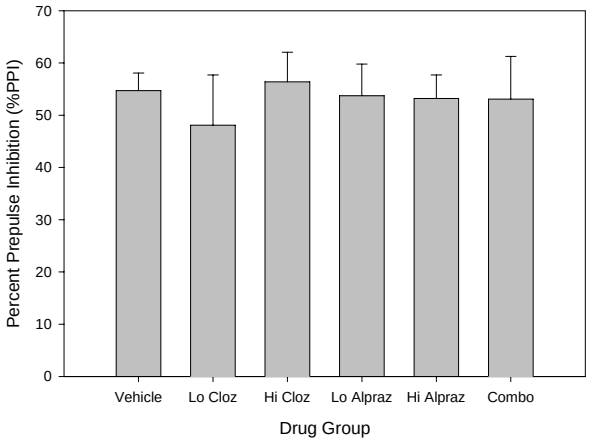
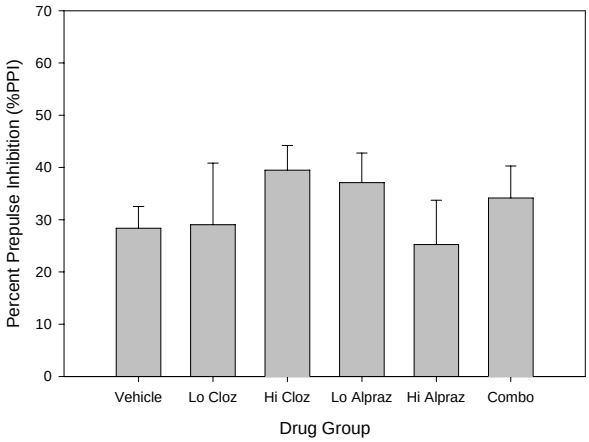


Figure 5. Ketamine



Figures 4-5. Percent prepulse inhibition to a prepulse of 82 dB and a startle stimulus of 110 dB in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 6. Saline

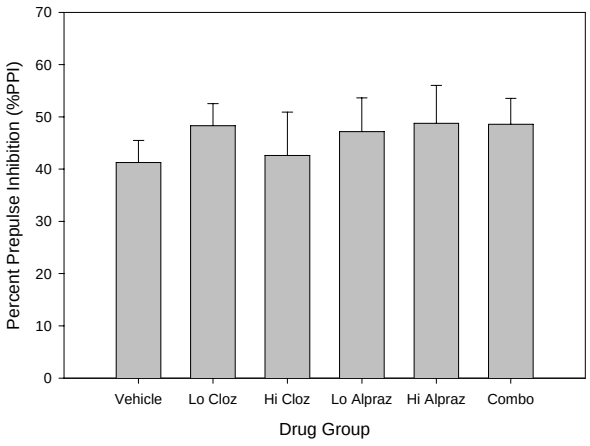


Figure 7. Ketamine

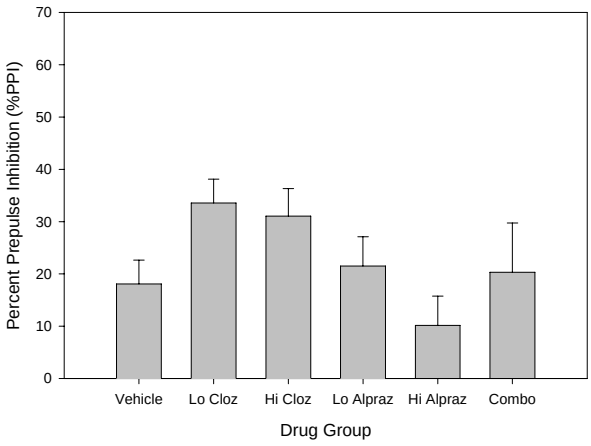


Figure 6-7. Percent prepulse inhibition to a prepulse of 68 dB and a startle stimulus of 120 dB in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 8. Saline

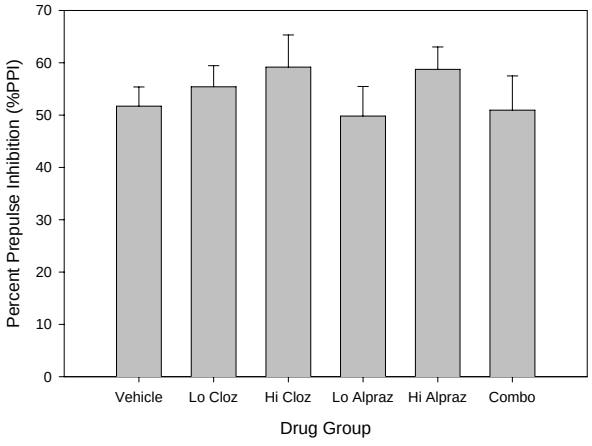
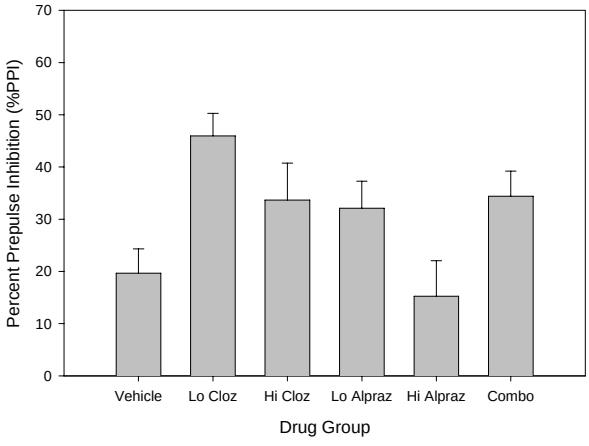


Figure 9. Ketamine



Figures 8-9. Percent prepulse inhibition to a prepulse of 82 dB and a startle stimulus of 120 dB in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Passive Avoidance

Passive avoidance is an example of a fear motivated avoidance test in which the animal must refrain from executing a previous response to avoid punishment, typically a low-voltage shock. Performance deficits in this measure may reflect interference with the ability to preserve information (Myhrer, 2003) and may index working memory and/or the ability to process information from short-term to long-term memory. Disruption of working memory is a common cognitive deficit in patients with schizophrenia (Fleming et al., 1997; Gold, Carpenter, Randolph, Goldberg, & Weinberger, 1997; Keefe, Lees-Roitman, & Dupre, 1997; Sullivan, Shear, Zipursky, Sagar, & Pfefferbaum, 1997). In the present experiment, this measure was used in an attempt to model ketamine-induced schizophrenia-like memory disruptions and to examine the effects of clozapine, alprazolam, and a combination treatment on these effects.

Training and testing latencies were compared using Wilcoxon Signed Ranks Tests (nonparametric paired t-tests) because latencies did not meet parametric criteria (data were not normally distributed and had heterogeneous variance) and were bounded by a maximum latency of 300 seconds. Planned comparison non parametric t-tests (Mann-Whitney t tests for independent samples) comparing animals treated with saline to animals treated with ketamine were performed for the training day and for the testing day. Training and testing latencies also were analyzed with Kruskal-Wallis Tests (nonparametric ANOVAs) for drug differences, also because latencies did not meet parametric criteria (data was not normally distributed and had heterogeneous variance) and were

bounded by a maximum latency of 300 seconds. Statistical values, degrees of freedom, and p values for each test are reported in Tables 6 & 7 in Appendix A.

Figures 10-13 present the latency to cross during training and testing. The top panel of each figure presents the responses of the saline-treated animals for each of the drug treatment groups. The bottom panel of each figure presents the responses of the ketamine-treated animals for each of the drug treatment groups.

All reported effects are significant at $p < 0.05$ unless otherwise noted. Results are reported and graphed for training and testing latencies for each group.

Task Validity: Training and testing latencies for each animal were compared to verify whether learning had occurred and to answer the question: did animals demonstrate memory for the aversive event (shock) following a 24 hour rest period? When all subjects were considered together, testing latencies were significantly longer than training latencies ($Z = -4.18$, $df = 143$), indicating that learning did take place. When the ketamine and saline groups were considered separately, saline animals also displayed significantly longer testing latencies than training latencies ($Z = -4.80$, $df = 72$), suggesting that learning occurred in these animals. There were no significant differences between training and testing latencies in the animals treated with ketamine, suggesting that learning was disrupted and did not occur.

Training: Planned comparison non parametric t-tests (Mann-Whitney t tests for independent samples) comparing latency to cross for animals treated

with saline to animals treated with ketamine were performed for the training day. There were no significant differences between these two groups, suggesting that ketamine did not affect training latencies.

Training latencies were examined non-parametrically using Wilcoxon-Signed Ranks because data did not meet criteria for parametric testing. When all animals were considered together on the training day, saline-treated animals had significantly longer training latencies than did ketamine-treated animals [$\chi = 3.90_{(1,144)}$]. There were no significant drug effects for either saline- or ketamine-treated animals on training latencies.

Testing: Planned comparison non-parametric t-tests (Mann-Whitney t tests for independent samples) comparing latency to cross for animals treated with saline to animals treated with ketamine were performed for the testing day. Ketamine significantly decreased latency to cross on the testing day, relative to saline controls ($Z = -3.94$, $df = 23$), suggesting that ketamine significantly disrupted learning relative to saline controls.

Testing latencies were examined non-parametrically using Wilcoxon-Signed Ranks because data did not meet criteria for parametric testing. Longer latencies on testing day are interpreted to mean learning has occurred, with greater latencies indicating greater learning. When all animals were considered together on the testing day, saline-treated animals had significantly longer testing latencies than did ketamine-treated animals [$\chi = 25.09_{(1,144)}$]. When subjects were divided by saline or ketamine treatment, there was no significant drug effect for ketamine-treated animals on testing latencies. For saline-treated animals,

there was a significant overall effect of drug [$\chi = 17.50_{(5,72)}$] to decrease testing latencies. Subsequent Mann-Whitney t-tests were performed comparing individual drug treatment groups with saline controls. Both dosages of clozapine decreased latency to cross on testing day relative to saline controls [clozapine 3.75 mg/kg (Z = -2.05, df = 23); clozapine 7.5 mg/kg (Z = -3.13, df = 23)]. Both dosages of alprazolam [alprazolam 0.75 mg/kg (Z = -3.29, df = 23) and alprazolam 1.5 mg/kg (Z = -2.86, df = 23)] also decreased latency to cross on the testing day relative to saline controls. The combination treatment also decreased latency to cross on testing day relative to saline controls (Z = -3.5, df = 23). These findings suggest that all drug treatments exerted a disruptive effect on learning in the passive avoidance measure.

Passive Avoidance Summary: Ketamine significantly disrupted learning (as measured by the passive avoidance task) relative to saline-treated controls. Clozapine, alprazolam, and the combination treatment all failed to significantly affect the ketamine-induced disruptions in learning. There were, however, significant drug effects on testing latencies for saline-treated animals such that all drug treatments disrupted learning in animals pre-treated with saline.

Figure 10. Saline

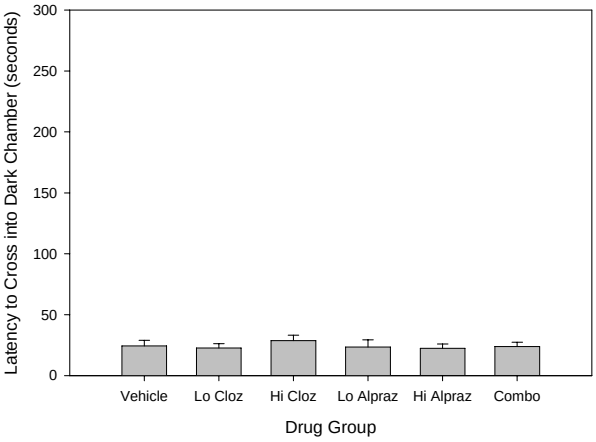
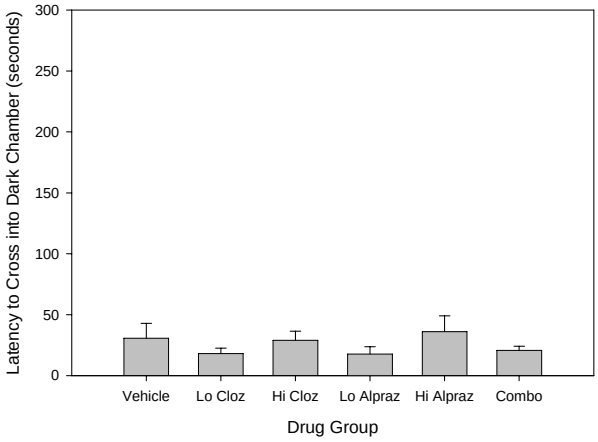


Figure 11. Ketamine



Figures 10-11. Latency to cross into the dark chamber during the **training** session in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 12. Saline

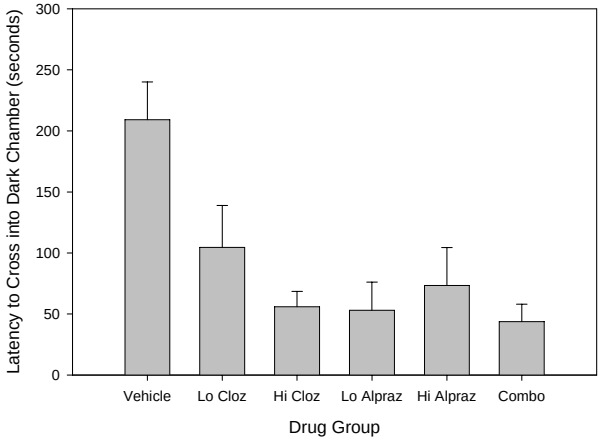
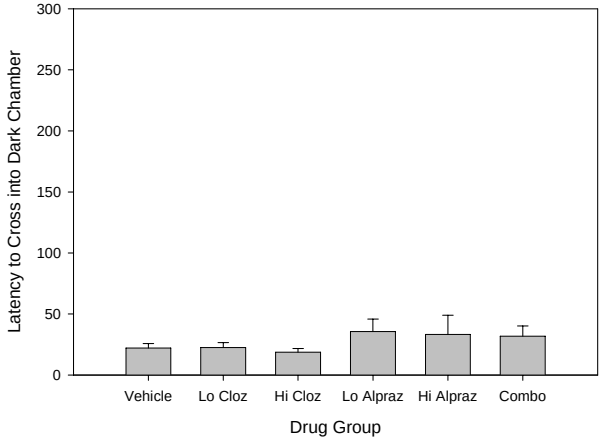


Figure 13. Ketamine



Figures 12-13. Latency to cross into the dark chamber during the **testing** session in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

CONFIRMATION OF HYPOTHESES – EXPERIMENT #1

Hypothesis 1: The hypothesis that ketamine administration would cause cognitive disruptions was **confirmed**.

Results: Ketamine-administration resulted in decreased PPI and impaired learning in the passive avoidance task, outcomes consistent with a disruption of cognitive function. These findings replicated reports of ketamine-induced PPI disruptions (Mansbach et al., 2001; Swerdlow et al., 1998) and ketamine-induced disruptions of learning (Babar et al., 1998; Gandolfi et al., 1990; Jones et al., 1990) in adult male Wistar rats.

Hypothesis 2: The hypothesis that clozapine would attenuate the cognitive disruptions caused by ketamine was **partially confirmed**.

Results: Clozapine, at the low dose (3.75 mg/kg), significantly attenuated the decreases in PPI caused by ketamine administration for certain startle and prepulse stimuli combinations. These findings replicated reports of restoration of ketamine-induced PPI disruptions by moderate dosages of clozapine (Mansbach et al., 2001; Swerdlow et al., 1998) and support the validity of ketamine administration as a model of certain cognitive disruptions in schizophrenia. Clozapine failed to attenuate the learning deficits caused by ketamine in the passive avoidance task, a novel finding that is discussed further in the Discussion section.

Hypothesis 3: The hypothesis that alprazolam would attenuate the cognitive disruptions caused by ketamine was **not confirmed**.

Results: Contrary to the hypothesis, alprazolam failed to attenuate the deficits cause by ketamine on PPI and on the passive avoidance task. The findings from this experiment failed to support the Braunstein-Bercovitz theory and the conceptual research model upon which this research is based. Implications and potential explanations are addressed in the Discussion section.

Hypothesis 4: The hypothesis that the combination treatment would attenuate the cognitive disruptions caused by ketamine was **not confirmed.**

Results: The combined treatment failed to attenuate the deficits cause by ketamine on PPI and on the passive avoidance task. Although clozapine, alone at a low dose, was able to attenuate the ketamine-induced deficits in PPI, a combination treatment of low-dose clozapine (3.75 mg/kg) and low-dose alprazolam (0.75 mg/kg) did not reverse ketamine-induced disruptions in either task.

DISCUSSION - EXPERIMENT #1

The sections below summarize the findings for each independent variable from Experiment #1. Possible explanations are then offered for the observed results. Next, relevant methodological issues and study limitations, specific to Experiment #1, are addressed. Possible implications for the current results and directions for future studies as well as general methodological issues and study limitations are discussed in the General Discussion section.

Prepulse Inhibition

Prepulse inhibition (PPI) of the acoustic startle response (ASR) is the process by which a weak, non-startling acoustic stimulus presented prior to a startling acoustic stimulus inhibits the startle response. Disruption of sensorimotor gating, specifically PPI, is a common cognitive deficit in schizophrenia (Braff, Grillon, & Geyer, 1992) and is commonly used as a model of schizophrenia-like cognitive disruptions in rodents (Geyer et al., 2001; Swerdlow & Geyer, 1998; Weiss & Feldon, 2001).

In the present experiment, administration of ketamine (8.5 mg/kg) significantly decreased % PPI in adult male Wistar rats relative to saline-treated controls to a variety of prepulse and startle stimuli. This finding is consistent with multiple reports of ketamine at similar dosages causing disruptions in prepulse inhibition (Brooks & Mansbach, 1997; DeBruin et al., 1999; Johansson et al., 1995; Mansbach & Geyer, 1991; Phillips & Grunberg, 2005; Swerdlow et al.,

1992). The disruption of PPI reported in this experiment also supports the use of ketamine as an animal model of sensorimotor gating deficits in schizophrenia.

Only clozapine (3.75 mg/kg) attenuated the ketamine-induced PPI disruptions. Significant attenuation of the disruptions by clozapine only occurred for one prepulse and stimulus combination (PP = 82 dB, Startle = 120 dB). These findings are consistent with previous studies (Mansbach et al., 2001; Swerdlow et al., 1998) that reported attenuation of ketamine-induced PPI deficits only in trials using prepulses of 76 dB or greater and startle stimuli of 117 dB or greater.

Determining the effects of medications successfully used to treat the human condition in an animal model of the condition is a common test of validity for animal models of specific disorders. Clozapine is an atypical antipsychotic that is considered to be the prototype atypical antipsychotic, and serves as a reference compound in the development of new, atypical antipsychotic medications. Clozapine successfully reduces both positive and negative symptoms in individuals suffering from schizophrenia (Stahl, 2002). The replicated finding, in previous reports (Mansbach et al., 2001; Swerdlow et al., 1998) and in the current experiment, that clozapine reverses or attenuates ketamine-induced PPI disruptions, suggests that the ketamine rat model of sensorimotor gating deficits in schizophrenia is a valid model to evaluate putative treatments for schizophrenia.

It is of interest to note that none of the drug treatments caused significant changes in PPI in saline-treated animals. This finding suggests that these drugs,

frequently used in human populations to treat a variety of psychotic and anxiety-related conditions, do not significantly disrupt or affect sensorimotor gating. Drug profiles with minimal side effects are desirable in all medications used in treating mental and other health disorders.

This experiment also was designed to test the theory proposed by Braunstein-Bercovitz and colleagues that the attentional disruptions commonly displayed by individuals with schizophrenia and schizophrenia-like disorders may not be specific symptoms of the disorder, but rather may be a result of the heightened anxiety experienced by these individuals (Braunstein-Bercovitz, 2000; Braunstein-Bercovitz, et al., 2002). These authors posited that the anxiety experienced by schizotypal and schizophrenic patients may be enough to induce cognitive disruptions, or it may aggravate existing cognitive deficits in these populations. To date, this interpretation had not been empirically evaluated in humans. This study is the first reported attempt to evaluate the theory in an animal model.

Based upon the proposed relationship between anxiety and the cognitive deficits associated with schizophrenia (Braunstein-Bercovitz, 2000; Braunstein-Bercovitz, at al., 2002), an alternative treatment for these deficits would be to medicate the cognitive deficits indirectly by using anxiolytic medications. Anxiolytics are better tolerated and cause fewer negative side effects than antipsychotic compounds. Based upon this research model, a reduction of anxiety would indirectly minimize or alleviate the cognitive deficits as well.

Contrary to the hypotheses for Experiment #1 and the working research model for this study, neither alprazolam nor the combination treatment (clozapine and alprazolam) attenuated the ketamine-induced disruptions in PPI. If the PPI deficits in this experiment were a result of ketamine-induced anxiety (as posited by Braunstein-Bercovitz) rather than a fundamental cognitive deficit, the anxiety-reducing medication alprazolam should have attenuated the PPI disruptions. This finding fails to support the proposed research model as well as Braunstein-Bercovitz's proposed relationship between anxiety and the cognitive deficits associated with schizophrenia.

There are two assumptions that must be explored before making a final judgment regarding the worth and validity of the Braunstein-Bercovitz theory and the conceptual research model for this study. First, it is assumed that ketamine is producing an increase in anxiety-like behaviors that is playing a role in, or causing, the disruption of PPI. As discussed in the Introduction, ketamine was chosen as the model for this study because it reportedly increases the appearance of anxiety-like behaviors in rodents (Hetzler & Waulet, 1985; Silvestre et al., 1998). To correctly evaluate the Braunstein-Bercovtiz theory and the validity of the conceptual research model, it must be established that ketamine is indeed increasing anxiety-like behaviors. Experiment #2 was designed, in part, to test the effects of ketamine on several measures of anxiety-like behaviors. The results of Experiment #2 are presented immediately following this section.

The second assumption to test the worth and validity of the Braunstein-Bercovitz theory and the conceptual research model for this experiment is that alprazolam reduces the ketamine-induced increases in anxiety like behaviors. Alprazolam reportedly decreases anxiety-like behaviors on a number of different measures of anxiety (File & Pellow, 1985; Griebel, Sanger, & Perrault, 1996; Hascoet & Bourin, 1998; Martin, Ballard, & Higgins, 2002; Prunell, et al., 1994), but it has not been tested in models using ketamine to induce anxiety-like behavior. To correctly evaluate the Braunstein-Bercovtiz theory and the validity of the conceptual research model, it must be established that alprazolam is indeed able to attenuate ketamine-induced increases in anxiety-like behavior. Experiment #2 was designed, in part, to test the effects of alprazolam on ketamine-induced anxiety-like behaviors on several measures of anxiety-like behaviors. The results of Experiment #2 are presented immediately following this section.

Should either of these assumptions be proven incorrect in Experiment #2, the validity of this study as an evaluation of the Braunstein-Bercovitz theory and the conceptual research model is questionable.

Passive Avoidance

Passive avoidance is a fear motivated avoidance test in which the animal must refrain from executing a previous response to avoid punishment. Performance deficits in this measure may reflect interference with the ability to preserve information (Myhrer, 2003). Learning deficits on the passive avoidance

measure may reflect a disruption of working memory, a common cognitive deficit in patients with schizophrenia (Fleming et al., 1997; Gold, Carpenter, Randolph, Goldberg, & Weinberger, 1997; Keefe, Lees-Roitman, & Dupre, 1997; Sullivan, Shear, Zipursky, Sagar, & Pfefferbaum, 1997). In the present experiment, this measure was used in an attempt to model ketamine-induced schizophrenia-like memory disruptions and to examine the effects of clozapine, alprazolam, and a combination treatment on these effects.

In the present research, ketamine (8.5 mg/kg) significantly disrupted learning (as measured by latency to cross on the testing day in the passive avoidance task) in adult male Wistar rats, relative to saline-treated controls. This finding is consistent with previous reports that ketamine interferes with learning on the passive avoidance measure in adult male rats (Babar, Ozgunen, Melikov, & Gaibova, 1998; Gandolfi, Dall'Olio, Roncada, & Montanaro, 1990; Jones, Bauerle, & DeNoble, 1990). The finding that ketamine, at dosages that successfully cause sensorimotor gating deficits (disrupted PPI), also appears to interfere with working memory suggests that ketamine may be an option for modeling multiple types of cognitive deficits seen in schizophrenia concurrently.

Contrary to the hypotheses, none of the drug treatments significantly affected the ketamine-induced disruptions in learning. Both dosages of clozapine (3.75 and 7.5 mg/kg), both dosages of alprazolam (0.75 and 1.5 mg/kg), and the combination treatment all produced significant disruptions in learning in animals that did not receive ketamine. The finding that clozapine (3.75 and 7.5 mg/kg) disrupts learning on the passive avoidance paradigm in rats

is a novel finding that is consistent with reports of similar disruptions caused by clozapine administration in mice (Ninan & Kulkarni, 1996; Rasmussen et al., 2001). The finding from the present research that alprazolam (0.75 and 1.5 mg/kg) interferes with learning in the passive avoidance measure replicates previous reports of alprazolam disrupting learning in the measure in mice and rats (Nishimura, Hata, Kawabata, Itoh, & Kita, 1989; Zivkovic, et al., 1995).

Given that both clozapine and alprazolam independently disrupted learning on the passive avoidance measure, it is not remarkable that they were unable to reverse the ketamine-induced learning deficits. As discussed previously, a common test for the validity of animal models is the ability of drugs that successfully treat the human condition to also alleviate modeled symptoms in animals. The failure of clozapine to reverse the learning disruptions caused by ketamine may indicate questionable validity for the ketamine model as it applies to schizophrenia-like working memory deficits. This interpretation should be tempered though by a lack of consistent evidence that clozapine is effective in reversing working memory deficits in human populations suffering from schizophrenia (Meltzer & McGurk, 1999). The working memory deficits common in schizophrenia may be mechanistically separate from sensorimotor gating deficits and may need to be medicated differently.

The finding that clozapine, alprazolam, and the combination treatment all independently disrupted learning in saline and ketamine-treated animals is of concern, given their common use as treatments for a variety of psychotic and anxiety disorders. These findings suggest that these medications may have

disruptive effects on learning and memory, although not on sensorimotor gating as measured by prepulse inhibition, in human populations. Learning and memory disturbances are potentially important negative side effects of treatment with these medications.

As previously discussed, to appropriately evaluate the Braunstein-Bercovitz theory and the conceptual research model for this study, it must be established that: (1) ketamine is indeed increasing the presence of anxiety-like behaviors in the subjects, and (2) alprazolam attenuates increases in these anxiety-like behaviors caused by ketamine administration. Experiment #2 was designed to address these two issues and to study the effects of clozapine and the combination treatment on ketamine-induced anxiety-like behaviors.

Research Design and Methods Relevant to Experiment #2

Subjects and Housing

Subjects were 144 adult male Wistar rats (Charles River Laboratories) (different from the rats used in Experiment #1). All animals were single-housed in standard rat cages (42.5 x 20.5 x 20 cm) on hardwood chip bedding (Pine-Dri) with continuous access to food (Harlan Teklad 4% Mouse/Rat Diet 7001) and water. Animals were single-housed to prevent effects or group housing in the social interaction measure. The housing room was maintained at room temperature (65 - 78°F) with a humidity of approximately 50% and a 12- hour reverse light cycle (lights on at 1900 and off at 0700 hours). Behavioral procedures were conducted during the dark portion of the light cycle, between 0800 and 1500 hours. This experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Pub, 82-23, rev. 1985).

Drugs

The drugs and drug-related procedures were identical to those used in Experiment #1.

Procedure

The data for Experiment #2 were collected over approximately 2 months (Please see **Table 4** for a detailed timeline of Experiment #2). Subjects were run in balanced groups of 18 to allow for sufficient time to complete each behavioral measure during the dark, or active, period of the subjects' light cycle. There was no handling period because handling has been shown to alter the effects of some drugs on rat behavior on the elevated plus maze (Andrews & File, 1993). After one or two rest days depending on group, rats were tested on the elevated plus maze apparatus following treatment with a vehicle control, clozapine, alprazolam, or combination treatment, and saline or ketamine. Open field activity was measured in rats following treatment with a vehicle control, clozapine, alprazolam, or combination treatment, and saline or ketamine on day 7 or day 8. Rats were tested in the social interaction test following treatment with a vehicle control, clozapine, alprazolam, or combination treatment, and saline or ketamine on day 11 or day 12.

Table 4. Experiment #2: Experimental Timeline

Day	**Group A**	**Group B**
1	Rats Arrive (n=18)	Rats Arrive (n=18)
2		
3	EPM Test	
4		EPM Test
5		
6		
7	Open Field Test	
8		Open Field Test
9		
10		
11	Social Inter. Test	
12		Social Inter. Test

EPM = Elevated Plus Maze

<u>Elevated Plus Maze:</u> Behavior on the elevated plus maze (EPM) was tested on day 3 or 4. Two arms of the elevated plus maze were "open" (without

any walls or barriers) and two arms were closed (enclosed on three sides by opaque black walls, leaving access to the center area). Following treatment with vehicle control, clozapine, alprazolam, or combination treatment, and saline or ketamine, rats were allowed to explore the maze for 5 minutes. Behaviors were videotaped via closed circuit TV camera for later scoring by two scorers. Behaviors scored were: percent time spent in the open arms and number of closed arm entries. Our laboratory has regularly used these procedures to examine the effects of various drugs and stress on EPM activity (Cook, 2001; Elliott et al., 2004).

Open Field Activity: Open field responses were measured on day 7 or 8. Open field activity was collected using an Omnitech Electronics Digiscan infrared photocell system [Test box model RXYZCM (16 TAO); Omnitech Electronics, Columbus, OH] in 40 cm x 40 cm x 30 cm Plexiglas arenas. Data were automatically gathered and transmitted to a computer. The apparatus monitored animal activity continuously for a total testing period of 1 hour, collecting data in 5 minute bins. Several activity-related variables were examined, including total horizontal activity, total vertical activity, and time spent in the center of the arena. These are procedures that have been performed extensively in our laboratory when examining the effects of various drugs and stress on locomotor activity (Cook, 2001; Elliott, et al., 2004; Faraday et al., 2003).

Social Interaction Test: Social interaction was evaluated on day 11 or 12. Animals were matched for weight within their drug treatment group to determine interaction pairs. The 10 minute interaction occurred in a 40 cm x 40 cm x 30 cm Plexiglas arena that was not the home cage. The interaction was videotaped and later scored by two trained scorers. Random checks of scoring accuracy were made by a second, trained scorer to verify that there was an inter-rater reliability of 0.90 or greater for all behaviors scored. Scored behaviors were: total time spent interacting and individual interaction behaviors, including touching, grooming, and sniffing other, as well as vertical activity (rears). These parameters and behaviors have been used in previous studies in this laboratory (Scheuefele et al., 2000) and in the published literature (File & Seth, 2003).

Statistical Issues

Data Analyses: Multivariate analyses of variance (MANOVA) were used for each measure of anxiety because there were several dependent variables within each measure that were significantly related. Significant between-subjects effects or interactions were further analyzed by splitting the data by the significant factor(s) and performing individual ANOVAs. *Post hoc* Dunnett's t tests were run when necessary. In addition, planned comparisons (t-tests) were made between animals treated with saline and animals treated with ketamine, based on Hypothesis #1 for each experiment. These planned comparisons were restricted to the experimental groups receiving ketamine or saline alone (*i.e.,* without clozapine or alprazolam) to clearly determine whether ketamine disrupted

the behavior that was measured. These analyses complemented the statistical analyses that included all treatment groups. All tests were two-tailed and an α level of 0.05 was used to determine statistical significance.

Sample Size: The sample size (cell size of n = 12) was determined in two ways: (1) using an approximation of the n used in published reports using the same behavioral measures and the same drugs or types of drugs (Geyer et al., 2001; Silvestre et al., 1998; Swerdlow et al., 1998), and (2) a power analysis based on pilot work performed in preparation for this research.

The power analyses were conducted following the procedures of Keppel (1991), Keppel, Saufley, and Tokunga (1992), and Cohen (1988). Using the effect sizes (omega squared) obtained in preliminary studies with the same behavioral measures and drugs used in the current research, calculations to achieve a power of 0.80, as recommended by Cohen (1988), reveal that the number of animals necessary is 12 per treatment cell. These empirically-based and calculated sample sizes also are consistent with studies that we have conducted with other medications and their effects on the behavioral measures that will be used in these experiments (Acri et al., 1995; Cook, 2001; Elliott et al., 2004; Faraday et al., 2003).

RESULTS - EXPERIMENT #2

Elevated Plus Maze

The EPM is one of the most commonly used measures of anxiety and anxiety-like behaviors in rodents (Belzung & Griebel, 2001; Bourin, 1997; File, 1987; Hogg, 1996; Rodgers et al., 1997). Percent time spent in the open arms and percentage of open arm entries are used to index anxiety and percentage of closed arm entries is used as a general index of activity (Fernandes & File, 1996; Hogg, 1996; Rodgers & Dalvi, 1997).

Planned comparisons (t-tests) were made between animals treated with saline and animals treated with ketamine (based on Hypothesis #1 for this experiment). A multivariate analyses of variance (MANOVA) was used to analyze EPM performance (time spent in the open arms, percentage of entries into the closed arms, percentage of entries into the open arms). Individual analyses of variance (ANOVAs) were used to determine the contributions of the individual variables (ketamine and drug treatment). Dunnett's t-test *post hoc* analyses were performed to compare individual treatment groups to the control group.

All reported effects are significant at $p < 0.05$ unless otherwise noted. Results are reported and graphed for time spent in the open arms, percent entries into the closed arms, and percent entries into the open arms.

Figures 14-19 present the individual indices of behavior measured on the elevated plus maze. The top panel of each figure presents the responses of the

saline-treated animals for each of the drug treatment groups. The bottom panel of each figure presents the responses of the ketamine-treated animals for each of the drug treatment groups. F values, degrees of freedom, and p values for each test are reported in Table 8 in Appendix A. The data for several animals that did not remain on the EPM for the entire 5 minute EPM session was excluded from the statistical analyses. Please see Table 9 in Appendix A for the adjusted *n*s for each treatment condition.

Planned comparison t-tests comparing animals treated with saline to animals treated with ketamine were performed for each of the EPM indices. Ketamine significantly decreased time spent in the open arms of the EPM [t(21)=4.23], but had no significant effects on percentage of entries into the open or closed arms relative to saline controls.

When all animals were considered together, there were multivariate ketamine effects [F(3, 111)=4.00]. Ketamine significantly decreased time spent in the open arms [F(1, 113)=11.30] and decreased the percentage of entries made into the open arms [F(1, 113)=4.63]. Ketamine had no significant effects on the percentage of entries into the closed arms, indicating that ketamine did not significantly affect the amount of locomotor activity.

When the ketamine and saline groups were considered separately, there was a significant overall drug effect in saline-treated animals, [F(15, 180)=2.26], but no drug effect in ketamine-treated animals. Within the saline group, there was a significant effect of drug on time spent in the open arms [F(5, 60)=7.27] and percentage of entries into the closed arms [F(5, 60)=2.97], and a trend for a

drug effect on percentage of entries into the open arms [F(5, 60)=2.12, p=0.075]. *Post hoc* analyses were performed on the EPM variables for saline-treated animals using Dunnett's t-tests. The high dose of clozapine (7.5 mg/kg) significantly decreased time spent in the open arms of the EPM relative to saline and vehicle-treated controls.

EPM Summary: There was a main effect of ketamine to significantly decrease time spent in the open arms and entries into the open arms, indicating an anxiogenic profile relative to saline-treated controls. There were no significant effects of clozapine, alprazolam, or the combination treatment in the ketamine-treated animals. The high dose of clozapine decreased time spent in the open arms of the EPM in saline-treated animals, suggesting a slight anxiogenic effect for clozapine.

Figure 14. Saline

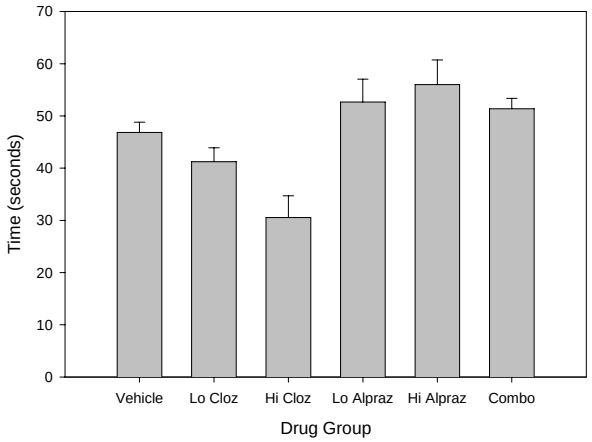
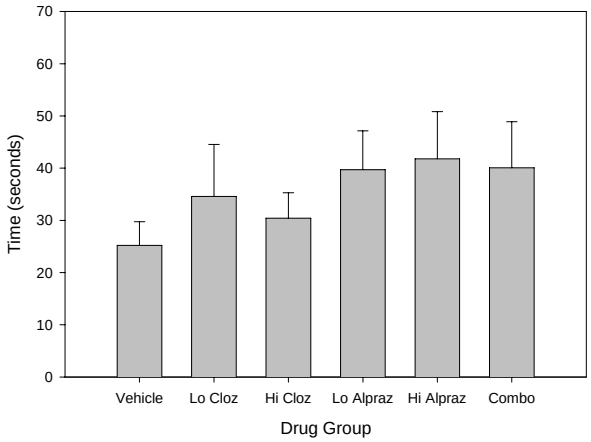


Figure 15. Ketamine



Figures 14-15. Time spent in the open arms of the EPM in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 16. Saline

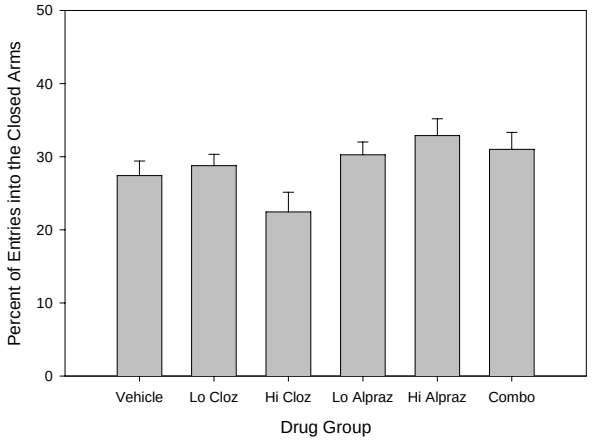
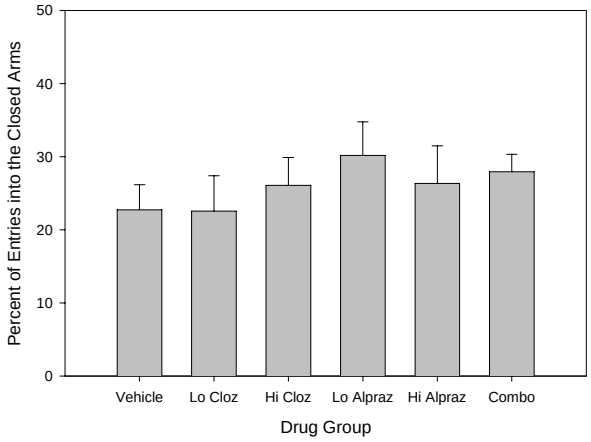


Figure 17. Ketamine



Figures 16-17. Percent of entries into the closed arms of the EPM in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 18. Saline

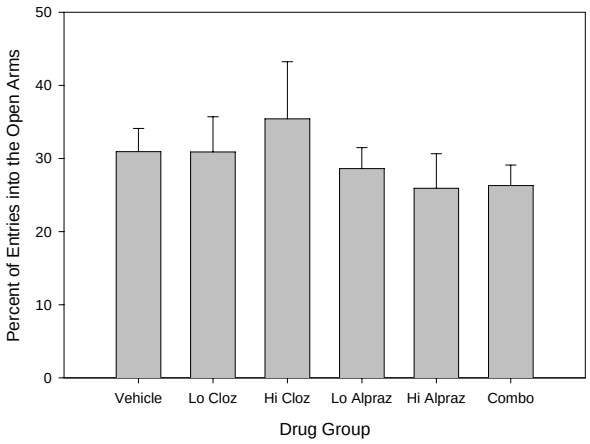
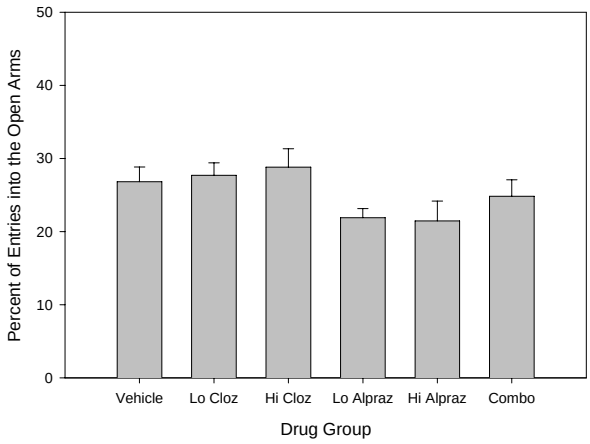


Figure 19. Ketamine



Figures 18-19. Percent of entries into the open arms of the EPM in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Open Field Locomotor Activity

There is a natural tendency of rodents to prefer the periphery of the arena and to spend a majority of the time ambulating close to the walls, a behavior known as thigmotaxis. Increased time spent in the center of the arena as well as increases in the ratio of center time to time in the periphery are considered indications of anxiolysis (Choleris, Thomas, Kavaliers, & Prato, 2001; Prut & Belzung, 2003). In addition, rearing (vertical activity) is also used as an index of exploring and anxiety-like behavior. A decrease in rearing is considered indicative of anxiogenic effects (Royce, 1977; Bhattacharya, Bhattacharya, & Ghosal, 1998).

Planned comparisons (t-tests) were made between animals treated with saline and vehicle control and animals treated with ketamine and vehicle control (based on Hypothesis #1 for this experiment) for each of the open field activity variables. A multivariate analysis of variance (MANOVA) was used to analyze activity in the open field (time spent in center of the arena, total horizontal activity, and total vertical activity). Individual analyses of variance (ANOVAs) were used to determine the contributions of the individual variables (ketamine and drug treatment). Dunnett's t-test *post hoc* analyses were performed to compare individual treatment groups with one another.

All reported effects are significant at $p < 0.05$ unless otherwise noted. Results are reported and graphed for time spent in the center of the arena, time spent rearing, and time spent moving.

Figures 20-25 present the individual indices of behavior measured in the open field locomotor activity measure. The top panel of each figure presents the responses of the saline-treated animals for each of the drug treatment groups. The bottom panel of each figure presents the responses of the ketamine-treated animals for each of the drug treatment groups. F values, degrees of freedom, and p values for each test are reported in Table 10 in Appendix A.

Planned comparison t-tests comparing animals treated with saline to animals treated with ketamine were performed for each of the open field indices. Ketamine significantly decreased time spent in the center of the arena [t(22)=2.27] and total vertical activity [t(22)=4.11], indicating an anxiogenic effect of ketamine. There were no significant differences between the groups on horizontal activity, suggesting that both groups were approximately equally active on this measure.

When all animals were considered together, there were significant multivariate effects of ketamine [F(3,130)=54.97] and drug [F(15, 396)=4.93], and a significant ketamine x drug interaction [F(15, 396)=3.92]. There was a significant effect of ketamine to decrease vertical activity [F(1, 132)=102.74], but no overall ketamine effects on time spent in the center of the arena or on horizontal activity. There were significant drug effects (the direction of the effects were different for the individual drugs – see below) for time spent in the center [F(5,132)=3.33], total horizontal activity [F(5,132)=12.99], and total vertical activity [F(5,132)=4.69]. Finally, there was a significant overall ketamine x drug

interaction for time spent in the center [F(5,132)=4.99] and total horizontal activity [F(5,132)=3.28].

Separate MANOVAs were performed on the ketamine and saline groups. There was a significant overall drug effect for both the saline [F(15, 198)=3.86] and the ketamine-treated animals [F(15, 198)=5.40]. For the saline group, drug status significantly affected time spent in the center of the arena [F(5,66)=5.44], total horizontal activity [F(5,66)=5.13], and total vertical activity [F(5,66)=2.95]. In ketamine-treated animals, there was a significant drug effect for time spent in the center of the arena, [F(5,66)=3.52], total horizontal activity [F(5,66)=9.62], and total vertical activity [F(5,66)=5.12].

Post hoc analyses (Dunnett's t-tests) were performed to determine individual treatment group differences on the open field variables for both the saline and the ketamine-treated animals. Relative to the saline-treated vehicle control group, animals treated with the high dose of clozapine (7.5 mg/kg) spent significantly less time in the center of the arena. The combination drug treatment significantly decreased total horizontal activity, and the high dose of alprazolam (1.5 mg/kg) significantly decreased both total horizontal activity and total vertical activity relative to the saline-treated, vehicle control animals. In ketamine-treated animals, only the combination treatment significantly increased time spent in the center relative to the vehicle control group. Horizontal activity was decreased in ketamine-treated animals that received the low (0.75 mg/kg) or high dosages (1.5 mg/kg) of alprazolam and the animals administered the combination treatment relative to vehicle-treated control animals. All drug groups in the ketamine-

treated condition displayed less vertical activity than did vehicle-treated ketamine controls.

Open Field Locomotor Summary: As hypothesized, ketamine significantly decreased time spent in the center of the arena and total vertical activity relative to saline-treated controls, suggesting an anxiogenic profile for ketamine. In ketamine-treated animals, only the combination treatment attenuated the reduction in time spent in the center. This attenuation was most likely because of the general activity disrupting effects of the combination treatment and not a true attenuation of the ketamine-induced anxiety-like behavior. Both dosages of alprazolam and the combination treatment reduced overall horizontal activity which may have compromised the accuracy of the center time measurement. This finding is discussed further in the Discussion section. In animals pretreated with saline, the high dose of clozapine (7.5 mg/kg) decreased time spent in the center, suggesting a general anxiogenic effect of clozapine.

Figure 20. Saline

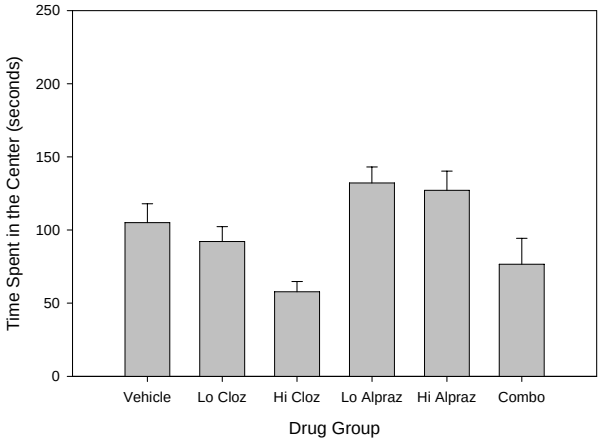
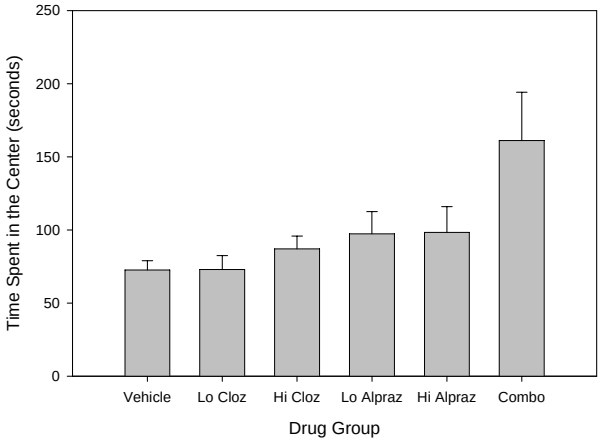


Figure 21. Ketamine



Figures 20-21. Time spent in the center of the locomotor activity chamber in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 22. Saline

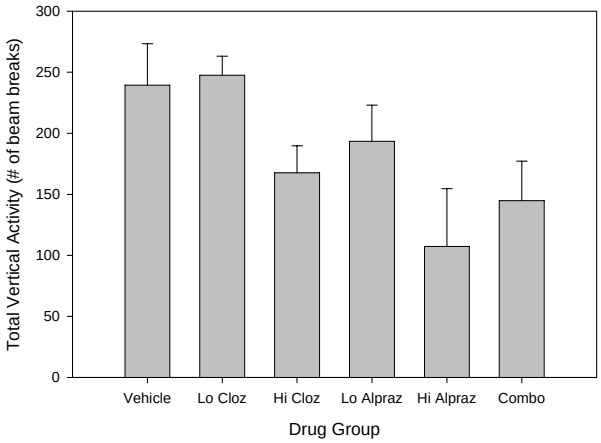
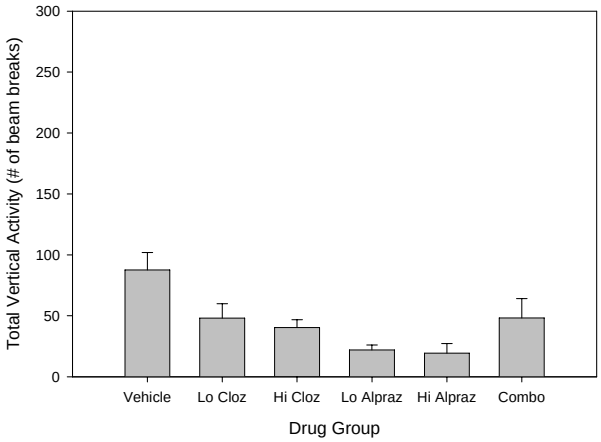


Figure 23. Ketamine



Figures 22-23. Total vertical activity in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 24. Saline

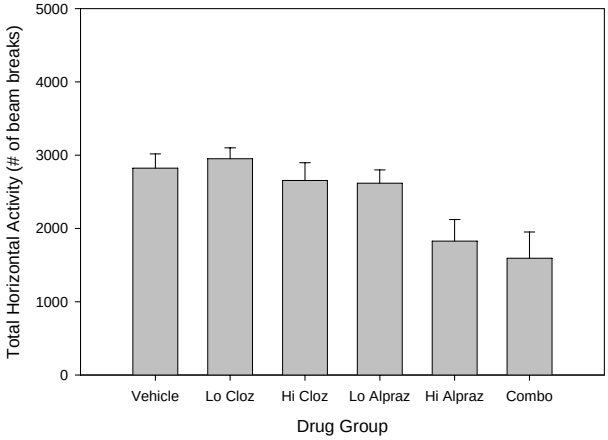
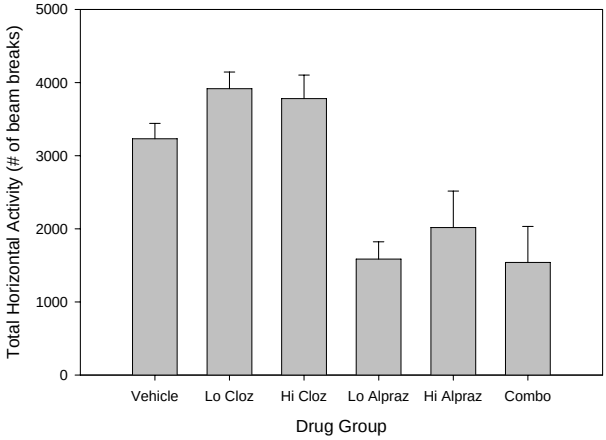


Figure 25. Ketamine



Figures 24-25. Total horizontal activity in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Social Interaction

As an index of anxiety, total time spent engaged in social interaction is quantified. A treatment-related increase in social interaction is indicative of anxiolysis, whereas a decrease in time spent in social interaction is considered to indicate anxiogenesis (File & Hyde, 1978; File & Seth, 2003).

Planned comparisons (t-tests) were made between animals treated with saline and animals treated with ketamine (based on Hypothesis #1 for this experiment) for each of the social interaction variables. A multivariate analysis of variance (MANOVA) was used to analyze social interaction variables (time spent in social interaction, time spent rearing, and time spent moving). Individual analyses of variance (ANOVAs) were used to determine the contributions of the individual variables (ketamine and drug treatment). Dunnett's t-test *post hoc* analyses were performed to compare individual treatment groups with one another.

All reported effects are significant at $p < 0.05$ unless otherwise noted. Results are reported and graphed for time spent in social interaction, time spent rearing, and time spent moving.

Figures 26 - 31 present the individual indices of behavior measured in interaction test. The top panel of each figure presents the responses of the saline-treated animals for each of the drug treatment groups. The bottom panel of each figure presents the responses of the ketamine-treated animals for each of the drug treatment groups. F values, degrees of freedom, and p values for each test are reported in Table 11 in Appendix A.

Planned comparison t-tests comparing animals treated with saline to animals treated with ketamine were performed for each of the open social interaction variables. Ketamine significantly decreased time spent in social interaction [t(22)=12.76], decreased time spent rearing [t(22)=12.73], and significantly increased time spent moving [t(22)=15.55], relative to saline controls.

A multivariate analysis of variance (MANOVA) was used to analyze overall ketamine and drug group effects on social interaction variables. There was a significant overall effect of ketamine [F(3, 130)=325.79], a significant overall effect of drug [F(15, 396)=6.07], and a significant ketamine X drug interaction [F(15, 396)=5.07]. Univariate ANOVAs revealed a significant ketamine effect for time spent in social interaction [F(1, 132)=287.96], time spent rearing [F(1, 132)=384.75], and time spent moving [F(1, 132)=107.63]. There was also a significant drug effect for time spent in social interaction [F(5, 132)=2.99], time spent rearing [F(5, 132)=2.42], and time spent moving [F(5, 132)=15.12]. Finally, there were significant ketamine x drug interactions for time spent in social interaction [F(5, 132)=3.61], time spent rearing [F(5, 132)=3.26], and time spent moving [F(5, 132)=8.15].

When ketamine and saline-treated animals were analyzed with separate MANOVAs, there were significant overall drug effects in both the saline [F(15, 198)=2.77] and the ketamine-treated animals [F(15, 198)=6.82]. In the saline condition, drug status significantly affected time spent in social interaction [F(5, 66)=2.69], time spent rearing [F(5, 66)=2.80], and time spent moving [F(5, 66)=2.51]. In the ketamine-treated animals, there was a significant drug effect for

time spent in social interaction [F(5, 66)=5.16], time spent rearing [F(5, 66)=3.45], and time spent moving [F(5, 66)=14.29].

Post hoc Dunnett's t-tests were performed to determine individual treatment group differences on the social interaction indices for both the saline and the ketamine-treated animals. In saline-treated animals, the high dose of clozapine significantly decreased time spent engaged in social interaction relative to saline and vehicle-treated controls. The high dose of alprazolam significantly decreased time spent rearing relative to saline and vehicle-treated controls. In the ketamine condition, the low dosage (0.75 mg/kg) of alprazolam significantly increased time spent in social interaction and there was a trend for the high dosage (1.5 mg/kg) to do the same. Low and high doses of alprazolam, as well as the combination treatment, decreased total time spent moving relative to the ketamine treated control group. The high dose of clozapine significantly decreased time spent rearing when compared to the ketamine and vehicle-treated controls.

Social Interaction Summary: As hypothesized, ketamine significantly decreased time spent in social interaction and time spent rearing relative to saline-treated controls, suggesting an anxiogenic profile for ketamine. Clozapine had no significant effect on the ketamine-induced anxiety-like behaviors, but alprazolam attenuated ketamine-induced reductions in time spent in social interaction. The combination treatment had no significant effect on these behaviors in ketamine-treated animals. In saline-treated animals, clozapine

significantly decreased time spent in social interaction, indicating an anxiogenic effect of clozapine consistent with the findings on the other measures.

Figure 26. Saline

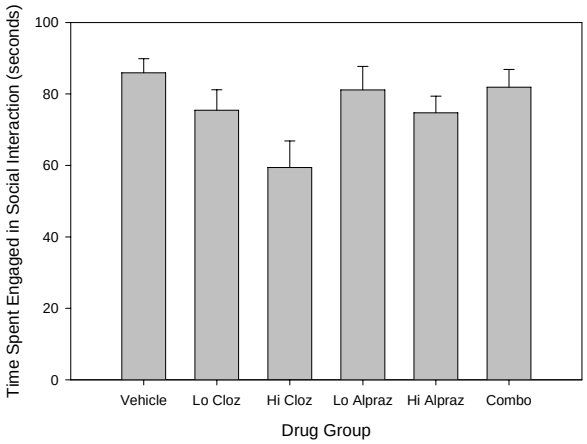
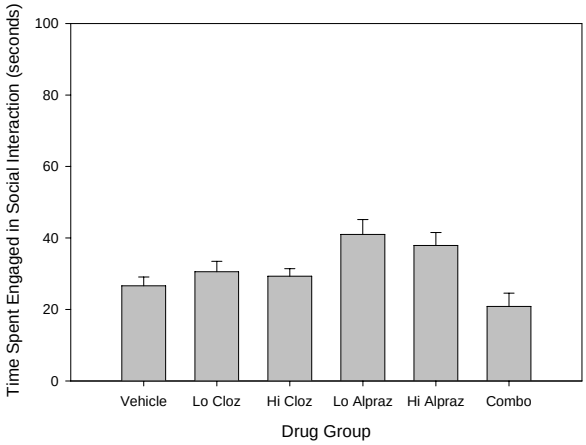


Figure 27. Ketamine



Figures 26-27. Total time spent engaged in social interaction in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 28. Saline

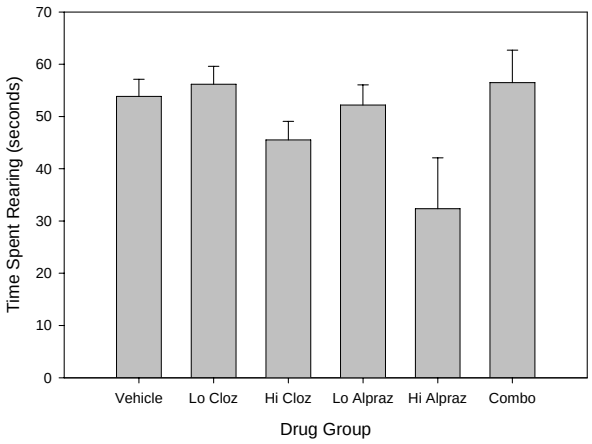
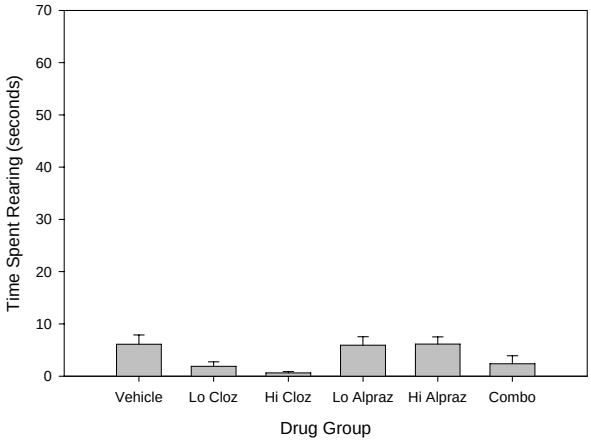


Figure 29. Ketamine



Figures 28-29. Total time spent rearing in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

Figure 30. Saline

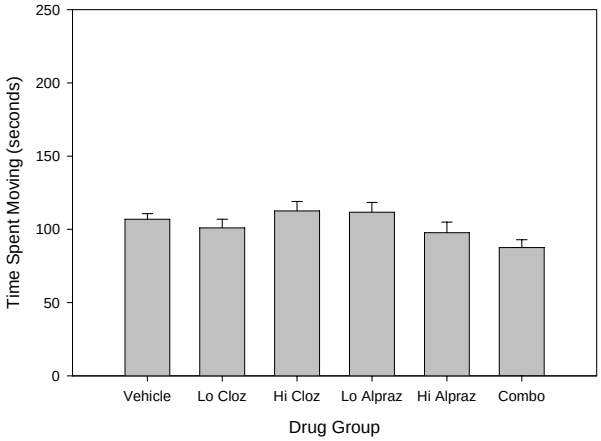
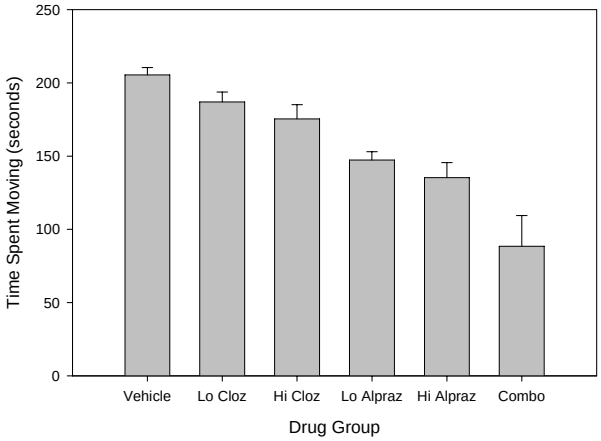


Figure 31. Ketamine



Figures 30-31. Total time spent moving in animals pretreated with saline or ketamine and drug vehicle, clozapine (3.75 or 7.5 mg/kg), alprazolam (0.75 or 1.5 mg/kg), or a combination treatment (3.75 mg/kg clozapine and 0.75 mg/kg alprazolam) (Means+SEM).

CONFIRMATION OF HYPOTHESES - EXPERIMENT #2

Hypothesis 1: The hypothesis that ketamine administration would increase anxiety-like behaviors was **confirmed**.

Results: Ketamine significantly increased anxiety-like behaviors on each of the anxiety measures in this study, a confirmation of the hypothesis. Ketamine significantly decreased time spent in the open arms and entries into the open arms of the elevated plus maze, indicating an anxiogenic profile for the drug. These findings replicated previous work by Silvestre and colleagues (1998). On the measure of open field locomotor activity, ketamine significantly decreased time spent in the center of the arena and total vertical activity in adult male Wistar rats, replicating and extending the work of Hetzler and Waulet (1985) in Long-Evans rats. The findings that ketamine decreased time spent in social activities and time spent rearing also indicate an anxiogenic profile and replicate and extend previously reported findings (Silvestre et al., 1998). Collectively, these findings suggest that ketamine administration does produce increases in anxiety-like behavior.

Hypothesis 2: The hypothesis that clozapine would have no effect on, or would increase, the anxiety-like behaviors caused by ketamine was **confirmed**.

Results: Despite producing anxiogenic effects in saline-control animals on all three measures of anxiety, clozapine had no significant effects on

measures of anxiety in animal pre-treated with ketamine, consistent with the hypothesis.

Hypothesis 3: The hypothesis that alprazolam would attenuate the increase in anxiety-like behaviors caused by ketamine was **partially confirmed**.

Results: Alprazolam significantly attenuated ketamine-induced anxiety-like behavior in the social interaction test, but had no significant effects on ketamine-induced anxiety-like behavior in the EPM or in the open field locomotor activity test. Alprazolam's test-specific capacity to reverse certain ketamine-induced anxiogenic behaviors is a novel finding and has potential relevance to the validity of ketamine as a model of comorbid symptoms of anxiety and schizophrenia. This issue is further addressed in the Discussion section.

Hypothesis 4: The hypothesis that the combination treatment would attenuate the increase in anxiety-like behaviors caused by ketamine was **not confirmed**.

Results: The combination treatment had no significant effects on the ketamine-induced increases in anxiety-like behaviors in the EPM and social interaction measures. While the combination treatment did result in a significant attenuation of center-time reductions in ketamine-treated subjects, this finding was the result of significantly disrupted general locomotor activity, rather than an anxiolytic effect of the treatment. This issue is addressed further in the Discussion section.

DISCUSSION – EXPERIMENT #2

The sections below summarize the findings for each independent variable from Experiment #2. Possible explanations are then offered for the observed results. Relevant methodological issues and study limitations, specific to Experiment #2, are addressed. Possible implications for the current results and directions for future studies as well as general methodological issues and study limitations are discussed in the General Discussion section.

Elevated Plus Maze

The EPM is one of the most commonly used measures of anxiety and anxiety-like behaviors in rodents (Belzung & Griebel, 2001; Bourin, 1997; File, 1987; Hogg, 1996; Rodgers et al., 1997). Indices of anxiety-like behaviors on the EPM include percent time spent in the open arms and percentage of open arm entries and percentage of closed arm entries is an index of general activity for the measure (Fernandes & File, 1996; Hogg, 1996; Rodgers & Dalvi, 1997).

In the present experiment, administration of ketamine (8.5 mg/kg) significantly decreased time spent in the open arms and entries into the open arms of the EPM in adult male Wistar rats relative to saline-treated controls. This finding is consistent with a previous report of ketamine at a slightly lower dosage (7.0 mg/kg) causing increases in anxiety-like behavior on the EPM measure in adult male Wistar rats (Silvestre et al., 1998). The increase in anxiety-like behaviors on the EPM supports a general anxiogenic effect of ketamine.

Contrary to the hypotheses, none of the drug treatments significantly affected the ketamine-induced anxiety-like behaviors. The low (3.75 mg/kg) and high (7.5 mg/kg) dosages of clozapine had no effect on ketamine-induced increases in time spent in the open arms and number of entries into the open arms. The high dosage of clozapine significantly decreased time spent in the open arms of the EPM, indicating an anxiogenic effect for clozapine in this measure. This finding is consistent with a report from a study using mice that the administration of low doses of clozapine (0.1 - 0.4 mg/kg) resulted in slightly less time spent in the open arms of the elevated plus maze relative to control animals (Manzaneque, Brain, & Navarro, 2002). Given the anxiogenic profile for clozapine on this measure, it is not remarkable that it was unable to attenuate ketamine-induced increases in anxiety-like behaviors. The combination treatment also failed to significantly affect EPM indices in ketamine-treated and saline-treated animals.

Neither the low (0.75 mg/kg) nor the high (1.5 mg/kg) dosage of alprazolam had a significant effect on ketamine-induced anxiety-like behaviors on the EPM. Additionally, there were no significant effects on the EPM for either dosage of alprazolam in saline-treated controls. This finding is in contrast to multiple reports that alprazolam at dosages identical or similar to those used in this study decreases the appearance of anxiety-like behaviors on the elevated plus maze measure (File & Pellow, 1985; Griebel et al., 1996; Martin et al., 2002; Prunell et al., 1994). It is possible that the rat strain is influencing alprazolam's effects on this measure. Whereas the present study used Wistar rats, the reports

of significant anxiolytic effects of alprazolam on the EPM were based on studies of Sprague-Dawley (Martin et al., 2002; Prunell et al., 1994) and hooded Lister (File & Pellow, 1985) rats. Strain is unlikely to explain the different findings because several studies have reported similar effects of benzodiazepines and benzodiazepine agonists on EPM indices in a variety of rat strains including Wistar, Lister, and Sprague-Dawley (Hogg, 1996).

It is noteworthy that the EPM is extremely sensitive to changes in a variety of different environmental conditions and variables. Variables such as amount of pre-test handling, running additional behavioral tests prior to EPM testing, housing conditions, lighting conditions, and differences in maze construction can significantly affect the outcome of elevated plus maze studies (Hogg, 1996). A small (3 cm) Plexiglas ledge was attached to the open arms of the EPM for this experiment to prevent animals from falling off the open arms. This was a particularly important addition given the high number of ketamine-treated animals that fell from the EPM during preliminary testing (approximately 60% of ketamine-treated rats did not complete the entire 5-minute EPM session because they fell from the open arms of the EPM). The addition of a ledge reportedly augments the anxiogenic effects of certain substances (Jones & Cole, 1994; Hogg, 1996), but reduces the anxiolytic effects of benzodiazepines, like alprazolam (Fernandes & File, 1996; Hogg, 1996). The addition of the ledge in this experiment maximized the number of subjects for which complete EPM data were available and potentially maximized the anxiogenic effects of ketamine.

However, it also may have reduced the anxiolytic effects of alprazolam to a statistically non-significant level.

The lack of significant effects for alprazolam in both saline- and ketamine-treated animals suggests questionable validity of the ketamine model of anxiety. Alprazolam is reportedly an effective treatment for a variety of anxiety disorders including generalized anxiety disorder (GAD) (Rickels & Rynn, 2002; Verster & Volkerts, 2004) and panic disorder, with and without agoraphobia (Kasper & Resinger, 2001; Verster & Volkerts, 2004), as well as non-anxiety disorders such as depression (Verster & Volkerts, 2004). If ketamine was producing anxiety-like behaviors in rats, as measured by the EPM, alprazolam should have attenuated or reversed the ketamine-induced increases in these behaviors.

It is possible that the EPM is not the best measure for studying the symptoms of anxiety that often co-occur with schizophrenia. Bourin (1997) suggests that the EPM, unlike many measures of anxiety-like behavior that measure more than one factor, is best suited to model panic disorder, and not generalized anxiety. A measure specific to panic disorder may not be suitable to study a model of schizophrenia that includes general symptoms of anxiety. The discussion for the additional behavioral measures of anxiety included in this experiment, open field locomotor activity and social interaction, follow.

Open Field Locomotor Activity

The open field test of locomotor activity can be used to index a variety of normal and abnormal behaviors. Of interest to the study of anxiety is a rodent's

natural tendency to prefer the periphery of the arena and to spend a majority of the time ambulating close to the walls, a behavior known as thigmotaxis. Increased time spent in the center of the arena as well as increases in the ratio of center time to time in the periphery are considered indications of anxiolysis (Choleris, Thomas, Kavaliers, & Prato, 2001; Prut & Belzung, 2003).

Rats treated with ketamine (8.5 mg/kg) spent significantly less time in the center of the arena and less time engaged in vertical activity relative to saline-treated controls. This finding replicates and extends a report by Hetzler and Waulet (1985) that high dosages of ketamine (50 mg/kg) produce an increase in locomotion in adult male hooded Lister rats that consists mainly of ambulation around the perimeter of the open field. As found in this experiment, even relatively low dosages of ketamine (8.5 mg/kg) can induce decreases in time spent in the center of the arena in adult male Wistar rats. The ketamine-induced decrease in center time and decrease in vertical activity are indicative of an anxiogenic profile for ketamine.

Clozapine (low dosage 3.75 m/kg or high dosage 7.5 mg/kg) failed to attenuate ketamine-induced decreases in center time. The high dosage of clozapine (7.5 mg/kg) also decreased time spent in the center of the arena in animals pretreated with saline. These findings suggest a slightly anxiogenic effect of clozapine, consistent with the hypotheses for this experiment.

Alprazolam (low dosage 0.75 mg/kg or high dosage 1.5 mg/kg) also failed to attenuate the ketamine-induced reduction in time spent in the center of the arena. Both the low and high dosages of alprazolam significantly decreased

general horizontal activity in ketamine-treated animals, whereas only the high dosage decreased general horizontal activity in saline-treated rats. Only the combination treatment (3.75 mg/kg clozapine + 0.75 mg/kg alprazolam) significantly attenuated the ketamine-induced decreases in time spent in the center of the arena. This combination treatment data should be considered with the findings that both dosages of alprazolam and the combination treatment reduced overall horizontal activity in ketamine and saline-treated animals. This reduction in general activity may have compromised the accuracy of the center time measurement in animals that received alprazolam and the combination treatment. The attenuation of ketamine-induced anxiety-like behaviors in the open field measure by the combination treatment is most likely attributable to decreases in general activity and, therefore, is not a true reduction in the anxiety-like behavior.

The failure to find significant effects of alprazolam in ketamine-treated animals also may be the result of general decreases in horizontal activity. A comparison of location specific behaviors in this measure (*e.g.,* time spent in the center) presumes equal activity among all groups. Group differences in general activity, such as those induced by alprazolam and combination treatments in this experiment, may hinder data interpretation for anxiety-like behaviors in the open field locomotion measure. Alternatively, the open field locomotor activity measure is reportedly more sensitive to anxiogenic effects (*e.g.,* the effects of ketamine and clozapine) than the anxiolytic effects that would be expected from a benzodiazepine, like alprazolam (Crawley, 1985). General use

benzodiazepines, including alprazolam, have produced mixed results on the open field locomotor activity measure, suggesting that it may not be a good measure for more general or varied symptoms of anxiety (Prut & Belzung, 2003). The open field test for anxiety-like behaviors may not be the best measure for testing a pharmacological model of concurrent schizophrenia and general symptoms of anxiety.

Social Interaction

Tests of social interaction can be used as an index of anxiety. Total time spent engaged in social interaction is quantified; a treatment-related increase in social interaction is indicative of anxiolysis, whereas a decrease in time spent in social interaction is considered to indicate anxiogenesis (File & Hyde, 1978; File & Seth, 2003).

Administration of 8.5 mg/kg ketamine resulted in significantly decreased time spent engaged in social interaction and significantly less time spent rearing relative to the saline-treated controls. The findings from this study replicate those previously reported by Silvestre and colleagues (1998). They demonstrated that the acute administration of 7 mg/kg ketamine has an anxiogenic effect on social interaction, decreasing time spent in social behaviors as well as central activity in the social arena. The increase in anxiety-like behavior in the social interaction test supports a general anxiogenic effect of ketamine.

Consistent with the hypotheses for this experiment, clozapine (3.75 mg/kg and 7.5 mg/kg) had no significant effect on ketamine-induced decreases in social

interaction. This finding replicates and extends previous reports of the effects of clozapine on social interaction and in combination with ketamine. Becker and Grecksch (2004) reported that 5.0 mg/kg clozapine had no affect on time spent engaged in social behaviors during a test of social interaction in animals pre-treated with 30 mg/kg ketamine, administered daily for 5 days. The current findings suggest that the lack of findings for clozapine applies to an acute ketamine administration model as well as to the repeated-acute or subchronic model used by Becker and Grecksch (2004). In saline-treated animals, clozapine significantly decreased time spent in social interaction, indicating an anxiogenic effect of clozapine.

Alprazolam attenuated the ketamine-induced decreases in social interaction, significantly at the low dosage (0.75 mg/kg) and with a trend for significance at the high dosage (1.5 mg/kg). Alprazolam had no significant effect on time spent in social interaction in animals that received saline. The finding that alprazolam alone had no significant effect on time spent engaged in social interaction is consistent with previous reports that alprazolam does not produce consistent anxiolytic effects in measures of social interaction (File & Pellow, 1985; Johnston & File, 1988). It is notable that alprazolam exerted anxiolytic effects and significantly attenuated ketamine-induced decreases in social interaction despite not causing anxiolytic effects when administered independently of ketamine. The finding that alprazolam attenuated the ketamine-induced decreases in social interaction is a novel finding and supports the use of ketamine as a model of certain anxiety-like behaviors in rats.

GENERAL DISCUSSION

The goal of this doctoral research was to examine the effects of a standard pharmacological treatment for schizophrenia, the antipsychotic clozapine, and a non-traditional pharmacological treatment, the anxiolytic alprazolam, as well as a combination of both treatment agents, on cognitive deficits and anxiety in a ketamine-induced rat model of schizophrenia. These studies examined the thesis that the cognitive deficits associated with schizophrenia can be medicated indirectly using anxiolytics, medications that are better tolerated than antipsychotic medications (see Figure 1 in the Introduction). In addition, these studies evaluated ketamine administration as a pharmacological model for comorbid symptoms of schizophrenia and anxiety.

Two separate experiments were conducted to address these goals. Experiment #1 examined the effects of clozapine, alprazolam, and a combination treatment on ketamine-induced cognitive disruptions in prepulse inhibition and passive avoidance. Experiment #2 examined the effects of clozapine, alprazolam, and a combination treatment on ketamine-induced anxiety-like behaviors on the elevated plus maze, open field test, and social interaction test.

In particular, the specific aims of the research were to determine: (1) the effects of ketamine on cognitive measures (*i.e.,* prepulse inhibition of the acoustic startle reflex (PPI) and passive avoidance); (2) the effect of drug treatments (*i.e.,* clozapine, alprazolam, and a combination treatment) on ketamine-induced cognitive disruptions; (3) the effects of ketamine on anxiety measures (*i.e.,*

elevated plus maze (EPM), open field locomotor activity, and social interaction); (4) the effect of drug treatments (*i.e.,* clozapine, alprazolam, and a combination treatment) on ketamine-induced anxiety-like behaviors.

The major findings of the study were: (1) Ketamine administration caused cognitive disruptions in PPI as well as passive avoidance; (2) Only clozapine attenuated the cognitive disruptions caused by ketamine, and only in the PPI measure; (3) Ketamine administration caused an increase in anxiety-like behaviors on the EPM, open field locomotor activity, and social interaction; (4) Only alprazolam decreased the ketamine-induced increases in anxiety-like behaviors, and only in the measure of social interaction. The implications of these findings are discussed in the following Discussion sections.

Evaluation of the Braunstein-Bercovitz Theory

Braunstein-Bercovitz and colleagues suggest that the attentional disruptions commonly displayed by individuals with schizophrenia and schizophrenia-like disorders may not be specific symptoms of the disorder, but rather may be a result of the heightened anxiety experienced by these individuals (Braunstein-Bercovitz, 2000; Braunstein-Bercovitz, et al., 2002). Based upon these reports and her own empirical studies, Braunstein-Bercovitz posited that the anxiety experienced by schizotypal and schizophrenic patients may be enough to induce cognitive disruptions, or it may aggravate existing cognitive deficits in these populations. The current research was designed to evaluate a conceptual research model based upon this theory. This model hypothesized

that, based on Braunstein-Bercovitz's theory, the cognitive disruptions in schizophrenia could be medicated indirectly with anxiolytic medications. To evaluate the model, the current research examined the effects of a standard pharmacological treatment for schizophrenia, the antipsychotic clozapine, and a non-traditional pharmacological treatment, the anxiolytic alprazolam, as well as a combination therapy, on cognitive deficits and anxiety in a ketamine-induced animal model of schizophrenia.

Alprazolam failed to attenuate cognitive disruptions in prepulse inhibition (PPI) and passive avoidance. Alprazolam did, however, attenuate the ketamine-induced anxiety-like responses in the social interaction measure, indicating that it was having an anxiolytic effect on at least one measure of anxiety-like behaviors. These findings fail to validate the conceptual research model and fail to support the Braunstein-Bercovitz theory regarding the relationship between cognitive disruptions and anxiety in schizophrenia.

Evaluation of the Ketamine Model of Schizophrenia and Anxiety

These studies also evaluated ketamine administration as a pharmacological model for comorbid symptoms of schizophrenia and anxiety. Although ketamine provides a proven model for the cognitive disruptions associated with schizophrenia and is reportedly anxiogenic, ketamine had not been validated as a pharmacologically-induced model of anxiety, alone or in combination with symptoms of schizophrenia.

Clozapine, an atypical antipsychotic used in the treatment of schizophrenia, effectively attenuated the ketamine-induced PPI deficits. This finding replicated multiple previous reports and suggests that ketamine is a valid model of the sensorimotor gating deficits associated with schizophrenia. Clozapine did not attenuate ketamine-induced disruptions in passive avoidance, a measure of working memory. This finding suggests that ketamine-administration may be limited to modeling specific cognitive disruptions associated with schizophrenia (*e.g.,* sensorimotor getting deficits, but not working memory deficits).

Alprazolam, an anxiolytic used to treat a wide variety of anxiety symptoms and disorders, did not attenuate ketamine-induced anxiety-like behaviors on the elevated plus maze (EPM) and open field test of locomotor activity. It did successfully attenuate ketamine-induced anxiety-like behaviors in the measure of social interaction. These findings collectively suggest that the ketamine model may be better suited to specific types of anxiety disorders or symptoms and is not a general model of anxiety.

Limitations/Future Research

There are several methodological limitations of the current research that may have affected the reported findings. The use of multiple cohorts to study cognitive function and anxiety with a single model is not ideal because it may introduce additional variance from inconsistencies among the cohorts. Multiple cohorts were required for the current research to maximize the number of

measures for each domain, two cognitive measures and three anxiety measures, while maintaining logistically manageable experiments. Given the findings that ketamine is most appropriate for the study of cognitive deficits as measured by PPI and anxiety as measured by social interaction, future studies could be limited to these two procedures and use a single experimental cohort. In addition, future studies should consider the pros and cons of counterbalancing the measures in order to avoid order effects but to maintain meaningful responses that are not confounded by other measures.

The current research was designed, in part, to evaluate the ketamine model of schizophrenia and anxiety; however, this model may not be the most appropriate for studying Braunstein-Bercovtiz's theory. The ketamine model, and other pharmacologically-induced models of schizophrenia, have been successful as tools to assess the efficacy of potential antipsychotic medications, but have rarely been used to examine theoretical constructs. One limitation of many pharmacologically-induced models of human disorders is that many of the drugs used as models of disordered behavior are non-specific in their effects and cause additional behavioral changes that make interpretation of the behavioral data difficult.

In addition, the issue of polypharmacy, the practice of administering multiple concurrent medications, may have interfered with some of the behavioral measures employed in this doctoral research project. As noted previously, ketamine is an anesthetic medication. Although it was administered in subanesthetic dosages, co-administration with alprazolam, a benzodiazepine

depressant, appeared to have synergistic depressant effects on locomotor activity and general mobility for several measures, including open field locomotion, social interaction, and the elevated plus maze (EPM). Decreased locomotion and mobility may have compromised the ability to interpret findings from these measures.

The latter two limitations, a pharmacologically-based model and negative behavioral effects from polypharmacy, could be minimized in future research by using a non-pharmacological model of schizophrenia and anxiety. The isolation-rearing model of schizophrenia is a non-pharmacological model of schizophrenia-like cognitive disruptions. Rats reared in social isolation from weaning exhibit PPI deficits that can be reversed by a variety of typical and atypical antipsychotics (Geyer et al., 2001). Although there are no reported studies of anxiety in this model, long-term social isolation in mice results in increases in anxiety-like and aggressive behaviors (Guidotti, et al., 2001). Future research should focus on validation of the neurodevelopmental isolation-rearing model as an alternative model to study concurrent symptoms of schizophrenia and anxiety without the interference of polypharmacy. Such a model may be more appropriate to screen for novel pharmacological treatments given the low likelihood of pharmacological interactions with the model.

The isolation-rearing model also may present a more suitable method to further evaluate the Braunstein-Bercovitz theory. It should be noted that the research upon which Braunstein-Bercovitz based her theory was conducted in a schizotypal population rather than a schizophrenic population. It is possible that

this particular theory may be more applicable to individuals suffering schizotypal symptoms rather than clinically diagnosable schizophrenia. If this is true, then a schizophrenia model of any variety may not be the best method for studying the theory.

Although the present research did not validate the Braunstein-Bercovitz theory, the failure to find supporting evidence may be the result of the methodological limitations rather than an empirical rejection of the theory. Given the high rate of comorbid schizophrenia and anxiety symptoms and the potential improvement in quality of life that alternative pharmacological therapies for the treatment of schizophrenia would offer, further study of Braunstein-Bercovitz theory is warranted. An alternative to the use of an animal model would be clinical research based on the same conceptual research model as the current research project that examines the effects of anxiolytic drugs on cognitive disruptions in schizophrenia. Although benzodiazepines are effectively used in connection with antipsychotics in schizophrenia (Pecknold, 1993), their effect on, and relationship to, cognitive disruptions is not well understood. This information is imperative to evaluate Braunstein-Bercovitz's suggestion that the attentional deficits in schizophrenia are a result of the heightened anxiety experienced by schizophrenics and not a symptom of the schizophrenia *per se*.

OVERALL SUMMARY AND CONCLUSIONS

This doctoral dissertation research project achieved its goal by examining the thesis that the cognitive deficits associated with schizophrenia can be medicated indirectly using anxiolytics, medications that are better tolerated than antipsychotic medications. The two experiments included in this research project evaluated the thesis that was based on a novel theory regarding the relationship between anxiety and the cognitive deficits associated with schizophrenia proposed by Braunstein-Bercovtiz and colleagues (Braunstein-Bercovitz, 2000; Braunstein-Bercovitz, et al., 2002). In addition, these experiments evaluated whether ketamine administration in rats would alter behaviors considered to model psychological symptoms of both schizophrenia and anxiety.

The results of this research project indicate that ketamine administration to rats may provide a useful model of the comorbidity of schizophrenia and anxiety. Specifically, ketamine alters prepulse inhibition of the acoustic startle reflex and alters social interaction in ways that are considered to be models of cognitive deficits and anxiety, respectively. Moreover, these effects of ketamine in rats were attenuated by clozapine (an atypical antipsychotic) and by alprazolam (an anxiolytic), respectively. However, it is noteworthy that the other behavioral indices of anxiety (*i.e.*, EPM, open field locomotor activity) that were increased by ketamine, were not attenuated by alprazolam. Therefore, the value of ketamine administration to model anxiety is limited.

With regard to the evaluation of the Braunstein-Bercovitz theory, the experiments failed to support this theory. However, the fact that the behavioral indices of anxiety in response to ketamine were limited, prevents reaching the unequivocal conclusion to reject the Braunstein-Bercovitz theory. It remains possible that the Braunstein-Bercovitz theoy applies to some aspects of anxiety that were not modeled by administration of ketamine. Further study of the relationship between anxiety and schizophrenia, using both animal models and human clinical trials, is necessary before drawing a firm conclusion about Braunstein-Bercovitz's theory. The proposed relationship between anxiety and the cognitive deficits associated with schizophrenia is intriguing and may provide novel options for medicating those suffering from schizophrenia and providing a higher quality of life.

Although it is disappointing that the results of this project did not clearly confirm or disconfirm the novel theory under study, this project was worthwhile for several reasons. Specifically, the findings provide further support for the notion that animal models are time effective, cost effective, and logistically feasible ways to study psychopathological conditions and possible treatments for these conditions. However, it is now clear that ketamine administration to rats only models some, and not all, aspects of anxiety. Therefore, other ways to model co-morbid schizophrenia and anxiety (*e.g.*, neurodevelopmental isolation rearing) need to be examined.

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APPENDIX A: TABLES

Table 5. Results of MANOVAs for Prepulse Inhibition Variables

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All Animals	**KETAMINE** 19.03 (4,129) p = 0.001	110 dB w/ 68 dB pp	47.27 (1,132)	<0.001
		110 dB w/ 82 dB pp	27.28 (1,132)	<0.001
		120 dB w/ 68 dB pp	45.41 (1,132)	<0.001
		120 dB w/ 82 dB pp	60.46 (1,132)	<0.001
	DRUG 0.91 (20,129) n.s.	110 dB w/ 68 dB pp	0.09 (5,132)	n.s.
		110 dB w/ 82 dB pp	0.55 (5,132)	n.s.
		120 dB w/ 68 dB pp	1.03 (5,132)	n.s.
		120 dB w/ 82 dB pp	2.23 (5,132)	0.055
	KETAMINE x DRUG 1.23 (20,129) n.s.	110 dB w/ 68 dB pp	0.28 (5,132)	n.s.
		110 dB w/ 82 dB pp	0.25 (5,132)	n.s.
		120 dB w/ 68 dB pp	1.28 (5,132)	n.s.
		120 dB w/ 82 dB pp	2.61 (5,132)	0.028
Ketamine	**DRUG** 1.31 (20,264) n.s.	110 dB w/ 68 dB pp	0.27 (5,66)	n.s.
		110 dB w/ 82 dB pp	0.58 (5,66)	n.s.
		120 dB w/ 68 dB pp	2.03 (5,66)	0.086
		120 dB w/ 82 dB pp	3.97 (5,66)	0.003
Saline	**DRUG** 0.74 (20,264) n.s.	110 dB w/ 68 dB pp	0.10 (5,66)	n.s.
		110 dB w/ 82 dB pp	0.18 (5,66)	n.s.
		120 dB w/ 68 dB pp	0.30 (5,66)	n.s.
		120 dB w/ 82 dB pp	0.62 (5,66)	n.s.

Table 6. Results of Wilcoxon Signed Ranks Tests for Passive Avoidance Variables

Group Tested	Effect	Z value (d.f.)	p value
All Subjects	Task Validity (Time)	-4.18 (143)	p<0.001
Saline	Task Validity (Time)	-4.80 (72)	p<0.001
Ketamine	Task Validity (Time)	-0.86 (72)	n.s.

Table 7. Results of Kruskal-Wallis Tests for Passive Avoidance Variables

Training			
Group Tested	**Effect**	**Chi Square (d.f.)**	**p value**
All Subjects	Ketamine	3.90 (1)	p<0.05
Saline	Drug	2.50 (5)	n.s.
Ketamine	Drug	3.54 (5)	n.s.
Testing			
Group Tested	**Effect**	**Chi Square (d.f.)**	**p value**
All Subjects	Ketamine	25.09 (1)	p<0.001
Saline	Drug	17.50 (5)	p<0.004
Ketamine	Drug	4.02 (5)	n.s.

Table 8. Results of MANOVAs for Elevated Plus Maze Variables

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All Animals	**KETAMINE** 3.99 (3,111) p = 0.010	Time Spent in Open Arms	11.30 (1,113)	<0.001
		Percent Open Arm Entries	4.63 (1,113)	0.034
		Percent Closed Arm Entries	2.28 (1,113)	n.s.
	DRUG 1.08 (15,339) n.s.	Time Spent in Open Arms	3.09 (5,113)	0.012
		Percent Open Arm Entries	1.55 (5,113)	n.s.
		Percent Closed Arm Entries	1.36 (5,113)	n.s.
	KETAMINE x DRUG 1.09 (15,339) n.s.	Time Spent in Open Arms	0.86 (5,113)	n.s.
		Percent Open Arm Entries	0.16 (5,113)	n.s.
		Percent Closed Arm Entries	0.77 (5,113)	n.s.
Ketamine	**DRUG** 0.77 (15,159) n.s.	Time Spent in Open Arms	0.59 (5,53)	n.s.
		Percent Open Arm Entries	0.76 (5,53)	n.s.
		Percent Closed Arm Entries	0.75 (5,53)	n.s.
Saline	**DRUG** 2.26 (15,180) p = 0.006	Time Spent in Open Arms	7.27 (5,60)	<0.001
		Percent Open Arm Entries	2.12 (5,60)	0.075
		Percent Closed Arm Entries	2.97 (5,60)	0.018

Table 9. Adjusted *n*s for the Elevated Plus Maze Variables

Saline	Original *n*	Adjusted *n*
Vehicle Control	12	11
Clozapine (3.75)	12	12
Clozapine (7.5)	12	12
Alprazolam (0.75)	12	11
Alprazolam (1.5)	12	10
Combination	12	10

Ketamine	Original *n*	Adjusted *n*
Vehicle Control	12	12
Clozapine (3.75)	12	11
Clozapine (7.5)	12	10
Alprazolam (0.75)	12	10
Alprazolam (1.5)	12	8
Combination	12	8

Table 10. Results of MANOVAs for Open Field Locomotor Activity Variables

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All Animals	**KETAMINE** 54.97 (3,130) p = 0.001	Time Spent in Center	0.01 (11,132)	n.s.
		Horizontal Activity	2.31 (11,132)	n.s.
		Vertical Activity	102.74 (11,132)	<0.001
	DRUG 4.93 (15,396) p = 0.001	Time Spent in Center	3.33 (5,132)	0.007
		Horizontal Activity	12.99 (5,132)	<0.001
		Vertical Activity	4.69 (5,132)	<0.001
	KETAMINE x DRUG 3.92 (15,396) p = 0.001	Time Spent in Center	4.99 (5,132)	<0.001
		Horizontal Activity	3.28 (5,132)	0.008
		Vertical Activity	1.67 (5,132)	n.s.
Ketamine	**DRUG** 5.40 (15,198) p = 0.001	Time Spent in Center	3.51 (5,66)	0.007
		Horizontal Activity	9.61 (5,66)	<0.001
		Vertical Activity	5.12 (5,66)	<0.001
Saline	**DRUG** 3.86 (15,198) p = 0.001	Time Spent in Center	5.44 (5,66)	<0.001
		Horizontal Activity	5.13 (5,66)	<0.001
		Vertical Activity	2.95 (5,66)	0.018

Table 11. Results of MANOVAs for Social Interaction Variables

Group Tested	Multivariate Effect and F value (d.f.)	Dependent Measure	Univariate F value (d.f.)	p value
All Animals	**KETAMINE** 325.79 (3,132) p = 0.001	Time Spent in Social Interaction	297.96 (1,132)	<0.001
		Time Spent Moving	107.63 (1,132)	<0.001
		Time Spent Rearing	384.75 (1,132)	<0.001
	DRUG 6.07 (15,396) p = 0.001	Time Spent in Social Interaction	2.99 (5,132)	0.014
		Time Spent Moving	15.11(5,132)	<0.001
		Time Spent Rearing	2.42 (5,132)	0.084
	KETAMINE x DRUG 5.07 (15,396) p = 0.001	Time Spent in Social Interaction	3.61 (5,132)	0.004
		Time Spent Moving	8.15 (5,132)	<0.001
		Time Spent Rearing	3.26 (5,132)	<0.001
Ketamine	**DRUG** 6.82 (15,198) p < 0.001	Time Spent in Social Interaction	5.16 (5,66)	<0.001
		Time Spent Moving	14.29 (5,66)	<0.001
		Time Spent Rearing	3.47 (5,66)	0.008
Saline	**DRUG** 2.77 (15,198) p = 0.001	Time Spent in Social Interaction	2.69 (5,66)	0.028
		Time Spent Moving	2.51 (5,66)	0.038
		Time Spent Rearing	2.80 (5,66)	0.023